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MINUTES OF THE 45th GENERAL ASSEMBLY OF THE EUROPEAN ASSOCIATION FOR THE STUDY OF DIABETES

held in Messe Wien, Vienna, Austria on 1 October 2009

Present:	**Dr. U. Smith**	**(President)**
	Dr. G. Spinas	**(Honorary Treasurer)**
	Dr. M. Stumvoll	**(Honorary Secretary)**
	Dr. E. Gale	**(Editor-in-Chief, Diabetologia)**
	Dr. J. Nolan	**(Chair, PGESC)**
	Dr.V. Jörgens	**(Executive Director)**
	Dr. M. Grüsser	**(Vice Director)**
	and 51 members	

The President welcomed everyone to the 45th General Assembly.

1. MINUTES 44th GENERAL ASSEMBLY 2008

Since there were no comments, the minutes were approved unanimously and officially signed as a correct record.

2. REPORTS

a) President

The President said he had given his report during the Presidential Address on Wednesday 30 September 2009.

b) Honorary Treasurer

Dr. Spinas reported that the accounts were in a healthy state. He said the main income came from the membership and registration fees. A transfer of 4 million Euros had been made to the Foundation.

Dr. Spinas expressed his thanks to Drs. Grüsser and Jörgens for their support and advice and to the team in Düsseldorf in general and to Mrs. Klee, Ms. Deparade and Ms. Weiss in particular for the precise handling of the accounts.

The President thanked the Honorary Treasurer for his diligence and asked if there were any questions. There were none.

c) Honorary Auditors

The President asked the Honorary Auditors, Drs. Paterson and Tack, to formally discharge the accounts. Dr. Tack confirmed that the accounts had been checked carefully and were in perfect order. Dr. Smith asked for the vote to accept the accounts.

The accounts were unanimously discharged (four abstentions).

d) Honorary Secretary

Dr. Stumvoll reported that more than 50% of those questioned in Rome scored the Meeting above average or outstanding. There had been a minor improvement in the ratings of the oral presentations.

For the Vienna Meeting, 2062 abstracts had been submitted and 1367 had been accepted. The highest number of submissions came from USA, UK and Germany. Regarding Travel Grants,146 had been awarded for the amount of €74,100.

Regarding the Stockholm Meeting, the Programme Committee had been put together and had had its first meeting. There are 16 in the Programme Committee, with one from USA. Dr. Stumvoll said he had received roughly 80 suggestions for symposia in Stockholm.

Dr. Stumvoll closed his report by thanking all members of the EASD staff, in particular Ms. H. Goliberzuch and

Mrs. M. Toledo, for their outstanding help and support with the organisation of the EASD Annual Meetings.

Dr. Smith thanked Dr. Stumvoll for his diligence and asked if there were any questions. There were none.

e) Editor-in-Chief, Diabetologia

Dr. Gale reported that the impact factor had improved, reaching the highest ever for Diabetologia, and the gap was closing on Diabetes Care. In 2008, 1694 articles had been submitted to Diabetologia; 51% went through full peer-review. The acceptance rate of 20.2% had increased slightly over 2007.

He expressed his thanks to the referees and associates and said their work was much appreciated. He also thanked his team in Bristol.

Dr. Smith thanked Dr. Gale for the excellent work he had done.

f) Chair, Postgraduate Education Sub-committee

Dr. Nolan reported that the postgraduate course in Kiev in April 2009 had been very successful and it was planned to return to the Ukraine in 2010. He thanked Dr. Boulton for the organisation of the extra-European courses in Vietnam, India and China.

Dr. Nolan reported that the web-based educational lectures were developing well and CME accreditation had been granted for these. He thanked Mr. Carey for his assistance.

Dr. Nolan thanked Dr. Czupryniak for his support as Secretary of the PGESC and the team in Düsseldorf, especially Mrs. Hata and Ms. Sommer, for their friendly assistance.

Dr. Smith thanked Dr. Nolan for his valuable work. There were no questions.

3. ELECTIONS

a) Vice President 2009-2012

The Council's election of Dr. F. Bosch was unanimously approved with one abstention.

b) Editor-in-Chief 2010-2013

The election of Dr. J. Zierath was unanimously approved with one abstention.

c) Honorary Treasurer Extension 2009-2010

The General Assembly unanimously approved Dr. Spinas' extension until 2010, with 1 abstention.

d) Chair PGESC, Extension 2009 -2010

The General Assembly unanimously approved Dr. Nolan's extension until 2010, with 1 abstention.

e) Council Members 2010-2013

The following Council Members were elected by the General Assembly:
Dr. Beguinot, F. (I) - unanimously;
Dr. Dekker, J. (NL) - 1 abstention;
Dr. Karpe, F. (UK) – unanimously;
Dr. Urbanavicius, V. (Li) - 1 abstention;

4. STUDY GROUPS

It was reported that two Study Groups had been disbanded:
Hypertension in Diabetes Study Group (HID)
Diabetes Optimization through Information Technology Study Group (DOIT)

5. HONORARY MEMBERSHIP

Drs. O. Crofford, J. Nerup and W. Waldhäusl were unanimously elected by the General Assembly.

6. ANY OTHER BUSINESS

There was no other business.

Dr. Smith thanked the out-going Vice President, Dr. C. Boitard, for his dedication and expressed his thanks for the confidence that Dr. Boitard had shown him as President.

Dr. Smith thanked the industry for their support. He also expressed his sincere gratitude to the Local Organising Committee for their outstanding contribution to the organisation of the 45th EASD Annual Meeting. He again thanked Dr. Stumvoll and the members of the Programme Committee for their hard work with regard to the scientific programme. The President warmly thanked the EASD team in Düsseldorf for their kind and efficient help.

Dr. Smith brought the General Assembly to a close at 19:00.

Agenda for the 46th General Assembly of the European Association for the Study of Diabetes

to be held in the Sutherland Hall at the Stockholmsmässan 18:00 on Thursday 23 September 2010

1. Minutes of the 45th General Assembly, Vienna, Austria 2009

2. Reports

a) President	Dr. U. Smith
b) Honorary Treasurer	Dr. G. Spinas
c) Honorary Auditors	Dr. K. Paterson Dr. C. Tack
d) Honorary Secretary	Dr. M. Stumvoll
e) Editor-in-Chief, Diabetologia	Dr. E. Gale
f) Chair, Postgraduate Education Subcommittee	Dr. J. Nolan
g) Chair, Extra-European Postgraduate Activities	Dr. A. J. M. Boulton

3. Elections

a) Extension, Honorary Treasurer (2010 - 2011)	G. Spinas
b) Honorary Secretary (2010 – 2013)	in place of M. Stumvoll
c) Chair, PGESC (2010 – 2013)	in place of J. Nolan
d) Council Members (2011 - 2014)	in place of T. BattelD. Matthews J. Philippe J. Zierath
e) Honorary Auditor (2010 - 2013)	in place of C. Tack

4. Study Groups

5. Honorary Membership

6. Any other business

46th EASD Annual Meeting of the European Association for the Study of Diabetes

Stockholm, Sweden, 20 – 24 September 2010

Abstracts

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PS 133 Cardiovascular biochemistry
PS 134 Liver, lungs and bone
PS 135 Steatohepatitis

OP 1 Novel formulations and delivery of insulin

1

Gene therapy for diabetic hyperglycaemia by expressing insulin and glucokinase in skeletal muscle: pre-clinical studies in diabetic dogs

D. Callejas[1,2], C.J. Mann[1], J. Montane[1], E. Ayuso[1,2], X. Leon[1,2], A. Andaluz[3], F. Mingozzi[4], K.A. High[4], F. Bosch[1,2];
[1]CBATEG and Department of Biochemistry and Molecular Biology, School of Veterinary Medicine, Universitat Autonoma de Barcelona, Bellaterra, Spain, [2]CIBER of Diabetes and Associated Metabolic Disorders, Barcelona, Spain, [3]Department of Medicine and Animal Surgery, School of Veterinary Medicine, Universitat Autonoma de Barcelona, Bellaterra, Spain, [4]Children's Hospital of Philadelphia, Abramson Research Center, Philadelphia, USA.

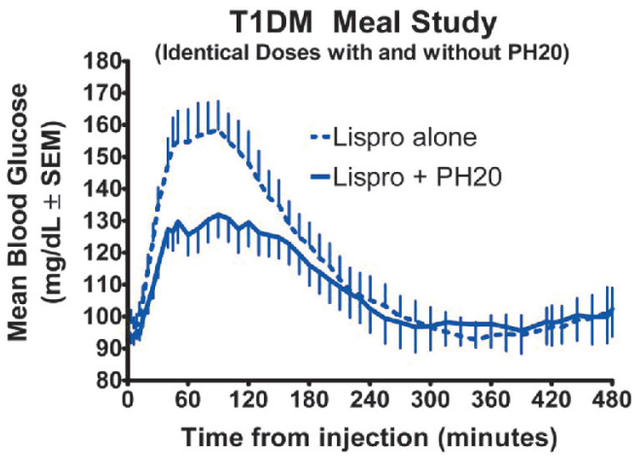
Background and aims: We previously demonstrated the feasibility of a potential gene therapy strategy for type 1 diabetes based upon engineering mouse skeletal muscle to co-express human insulin (hIns) and glucokinase (Gck). Both genes act synergistically such that local production of hIns improves glucose uptake, whereas Gck acts as a glucose sensor in a concentration-dependent manner to facilitate glucose disposal. We wished to perform a pre-clinical study in diabetic dogs to demonstrate the feasibility, efficiency, duration of effects as well as any biochemical and safety issues.
Materials and methods: Five dogs were made diabetic with streptozotocin and alloxan. Dog 1 was treated subsequently after diabetes induction with 2.5E12 vg/kg of AAV1-CMV-hIns. Dog 2 was given AAV1-CMV-hIns (1.0E12 vg/kg) only, whereas dogs 3 and 4 received AAV1-CMV-hIns and AAV1-CMV-Gck simultaneously (1.0E12 vg/kg each vector). In addition, Dog 5 was left as an untreated diabetic control for 8 months before receiving the same treatment as Dog 3.
Results: In Dog 1 human C-peptide was successfully detected in serum and associated with improved glucose disposal after oral glucose tolerance test (GTT) and a reduced fasting glycaemia in the absence of any negative response to the vector. Dog 1 was sacrificed at 3 weeks after the treatment and insulin expression was confirmed by qPCR and Northern Blot in skeletal muscle, but not in other tissues. In the other four additional dogs that were treated with lower doses we wanted to find the minimum effective dose of AAV1-CMV-hIns able to produce normoglycaemia in fasting conditions. Circulating levels of human C-peptide were detected for >3 years after injection in Dogs 2, 3 and 4 and for >2 years in Dog 4. Dog 2 showed a nearly normalized fasting glycaemia whereas Dog 3, 4 and 5 achieved completely normalized fasting glycaemia and a vastly improved ability to dispose of glucose compared to Dog 2 or Dog 4 when diabetic, by GTT, suggesting the concerted action of hINS and Gck. Dog 5 demonstrated a continuous deterioration in weight and biochemical parameters (AST and ALT) while diabetic which was immediately rectified upon treatment.
Conclusion: Taken together, these results suggest that engineering skeletal muscle to produce insulin and glucokinase may be a potential therapy for the treatment of type 1 diabetes, because it shows long term efficacy and safety.
Supported by: SAF 2008-00962 and the EC (FP6 CLINIGENE)

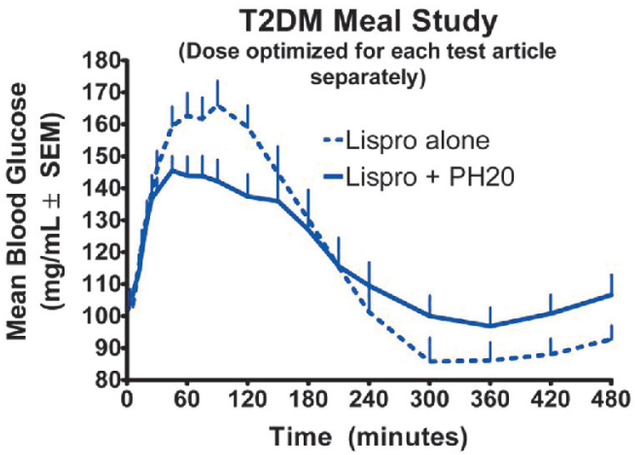
2

Improved glycaemic variability in type 1 diabetes mellitus and type 2 diabetes mellitus patients by coinjection of prandial insulin analogue with human hyaluronidase

M. Hompesch[1], D. Muchmore[2], L. Morrow[1], E. Ludington[2], D. Vaughn[2];
[1]Profil Institute for Clinical Research, Chula Vista, [2]Halozyme Therapeutics, Inc., San Diego, USA.

Background and aims: Studies in T1DM and T2DM patients to compare the postprandial glucose (PPG) response to a liquid meal for insulin lispro injected ± human hyaluronidase (PH20).
Materials and methods: Two standardized liquid meal studies were conducted; one in 22 T1DM patients [15 male, 9 female; mean age 40.7 (±10.7); mean BMI 24.2 (± 2.9)] and the other in 23 T2DM patients [14 male, 9 female; mean age 52 (37-69); mean BMI 33.5 kg/m2 (23.7-45.0)] on high insulin doses, mean daily dose 105U (60-230). Patients fasted (10h) and refrained from SC insulin (>9h) before dosing. 2h before a liquid meal (60g CHO for T1DM or 80g CHO for T2DM), patients were titrated to 110±20mg/dL glucose target with IV glucose and/or IV insulin followed by a 30min intervention-free period. For the T1DM study, an optimum dose was found for lispro+PH20 during up to 3 dose-finding visits (targeting a postprandial range of 60-160 mg/dL) and the same dose was studied for lispro alone, while for the T2DM study the optimum dose (targeting a postprandial range of 70-140mg/dL) was separately optimized for lispro±PH20 (from 3 dose-escalating test meal visits each). Lispro±PH20 was injected SC immediately pre-meal, and plasma insulin and glucose concentrations were monitored for 8h.
Results: PH20 coinjection reduced hyperglycemic excursions in both studies with the same (T1DM) or reduced (T2DM) hypoglycemic risk. In the T1DM study, peak PPG was reduced from 174 to 148 mg/dL (p=.002) and the total hyperglycemic excursions (AUC > 140 mg/dL) were reduced 79%, allowing more patients to reach PPG goal (91% +PH20 v. 55% for lispro alone met the ADA goal of ≤180mg/dL). In the T2DM study, peak PPG was reduced from 178 to 165 mg/dL (p=.095) and the total hyperglycemic excursions (AUC > 140 mg/dL) were reduced 44%, allowing more patients to reach PPG goal (71% +PH20 v. 48% for lispro alone met the ADA goal of ≤180mg/dL). Hypoglycemic risk was similar in the T1DM study with equal lispro doses, and reduced in the T2DM study where the optimum dose was reduced by 8% for lispro+PH20. All injections were well tolerated.
Conclusion: Lispro+PH20 provided superior control of postprandial blood glucose compared to lispro alone in patients with both T1DM and T2DM in these test meal settings.





3

30 month post trial follow up of HbA_{1c} with continuous intraperitoneal insulin infusion in type 1 diabetes

S.J.J. Logtenberg[1], N. Kleefstra[1,2], S.T. Houweling[2], K.H. Groenier[3], H.J.G. Bilo[1,4];
[1]Diabetes Centre, Isala Clinics, Zwolle, [2]Langerhans Medical Research Group, Zwolle, [3]General Practice, UMCG, Groningen, [4]Internal medicine, UMCG, Groningen, Netherlands.

Background and aims: Results from our randomized controlled trial (RCT) showed that with continuous intraperitoneal (IP) insulin infusion with an implantable pump (see Figure 1) it is possible to achieve better glycemic control and quality of life compared to subcutaneous insulin administration in patients with type 1 diabetes (T1DM). The aim of this analysis was to investigate patients therapy choice and glycemic control 30 months after the end of the trial.
Materials and methods: The 23 patients that ended the RCT in 2007/2008 all continued to use the IP pump. Last known HbA1c values were collected in the first quarter of 2010. Status regarding therapy mode were extracted from hospital records. Paired t-tests were used to compare HbA1c at the end of the IP study phase with mean HbA1c at follow up.
Results: In March 2010, 22 (12 female, 10 male) patients were still treated with CIPII, 1 patient (female) was back on subcutaneous insulin due to neuropathic pains, for which the patient blamed the IP pump. Mean age at follow up was 46.6 (12.0) years; mean diabetes duration at the start of the study was 22.6 (10.6) years; mean baseline HbA_{1c} 8.6 (1.1) %; HbA_{1c}>7.5% in 20 subjects; hypoglycemic events ≥5/week in 14 subjects. HbA1c was collected after 2.3 (0.6) years after the end of the study. Mean HbA1c was 7.7% (1.1). Compared to prestudy HbA1c values, this is a significant reduction of 0.83% (CI; -1.3, -0.4). Compared to the end of the IP phase of the trial, the results are comparable (0.2% (CI; -0.3, 0.7).
Conclusion: Our analysis shows that with CIPII it is possible not only to improve glycemic control in the short term, but also to achieve sustained improvement in glycemic control in patients with T1DM who were insufficiently controlled previously despite intensified subcutaneous insulin regimens. Furthermore, there is a low drop out rate with CIPII (1 out of 23 of patients).

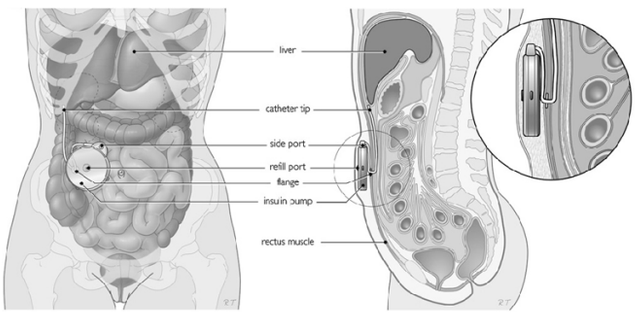


Figure 1: Schematic representation of the position of the insulin pump and catheter in situ.
Supported by: Medtronic

4

Insulin degludec, a new generation ultra-long acting insulin, used once daily or 3-times weekly in people with type 2 diabetes: comparison to insulin glargine

C. Mathieu[1], G. Fulcher[2], P.V. Rao[3], N. Thomas[4], L. Endahl[5], T. Johansen[5], A.J. Lewin[6], J. Rosenstock[7], M. Pinget[8], B. Zinman[9];
[1]UZ Gasthuisberg K.U.Leuven, Belgium, [2]Royal North Shore Hospital, University of Sydney, Australia, [3]Nizam's Institute of Medical Sciences University, Hyderabad, India, [4]Christian Medical College, Vellore, India, [5]Novo Nordisk A/S, Soeborg, Denmark, [6]National Research Institute, Los Angeles, [7]Dallas Diabetes and Endocrine Center at Medical City, Dallas, USA, [8]University Hospital Strasbourg, France, [9]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Canada.

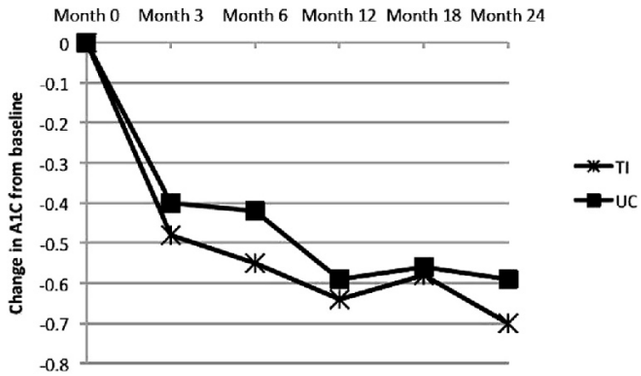
Background and aims: Insulin degludec (IDeg) is a novel insulin analog that forms soluble multi-hexamer assemblies after s. c. injection, resulting in ultra-long duration of action. The aim of this phase 2 trial was to investigate the efficacy and safety of IDeg formulations administered once daily (OD) or 3-times weekly (3TW) in insulin-naïve people with type 2 diabetes inadequately controlled on OADs.
Materials and methods: This was a 16-week, open-label, randomized, 4-arm, parallel-group, treat-to-target trial. Participants (mean: 54.2 years, HbA_{1c} 8.7%, fasting plasma glucose (FPG) 10.2 mmol/l, BMI 29.5 kg/m^2) received an OD formulation of IDeg (IDeg OD, n=60), a 3TW formulation of IDeg (IDeg 3TW, n=62), an alternative IDeg OD formulation (development discontinued, results not shown, n=61) or insulin glargine OD (IGlar, n=62), all in combination with metformin. All insulins were injected s. c. in the evening and titrated to achieve FPG 4.0-6.0 mmol/l. www.clinicaltrials.gov ID: NCT00611884.
Results: HbA_{1c} after 16 weeks of treatment was similar across treatment arms with regard to mean reduction from baseline (IDeg OD: -1.3%; IDeg 3TW: -1.5%; IGlar: -1.5%; p=NS for all pairwise comparisons) and final mean value (IDeg OD: 7.4%; IDeg 3TW: 7.3%; IGlar: 7.2%). Treatments were also comparable with respect to final mean FPG (IDeg OD: 6.3 mmol/l; IDeg 3TW: 6.5 mmol/l; IGlar: 6.4 mmol/l) and mean reductions from baseline (IDeg OD: -3.6 mmol/l; IDeg 3TW: -4.2 mmol/l; IGlar: -3.4 mmol/l). At end-of-trial, mean weekly insulin dose was similar for IDeg OD (3.1 U/kg/week=0.45 U/kg/day), IDeg 3TW (3.4 U/kg/week=0.49 U/kg/day) and IGlar (3.3 U/kg/week=0.48 U/kg/day). Body weight remained stable throughout the trial in all treatment arms. Rates of confirmed hypoglycemia (PG <3.1 mmol/l or requiring assistance) were low and only one severe event was reported (for IDeg 3TW). The rate of confirmed hypoglycemia appeared lower for IDeg OD than IDeg 3TW and IGlar (0.6, 2.3, 1.1 events/patient year, respectively; p=NS for both comparisons). The proportion of subjects with adverse events (AEs) was similar across treatment arms (IDeg OD: 47%; IDeg 3TW: 50%; IGlar: 66%) with no specific patterns or clustering. The majority of AEs were mild or moderate in severity.
Conclusion: This proof-of-concept trial demonstrated that IDeg used 3-times weekly or once daily was safe, well tolerated and provided similar glycemic control to IGlar.
Supported by: Novo Nordisk

5

Glycosylated hemoglobin and hypoglycaemia in patients with type 2 diabetes mellitus: Technosphere insulin and usual antihyperglycaemic regimen vs usual antihyperglycaemic regimen

A.H. Boss[1], P. Raskin[2], M. Phillips[1], A. Rossiter[1], P.C. Richardson[1];
[1]MannKind Corporation, Valencia, [2]University of Texas Southwestern Medical Center at Dallas, USA.

Background and aims: Technosphere Insulin (TI) is an ultra-rapid-acting inhaled insulin with a pharmacokinetic profile well suited for earlier control of postprandial plasma glucose. One objective of this trial was to compare the efficacy and safety of prandial TI with usual diabetes care (UC) in patients with type 2 diabetes mellitus (T2DM) with inadequate glycemic control (glocosylated hemoglobin [A1C] >6.6%, ≤12.0%).
Materials and methods: Patients either incorporated TI into their usual antihyperglycemic regimen (TI group; n=656), or continued their usual antihyperglycemic regimen, which could include insulin, oral hypoglycemic drugs, and/or diet and exercise (UC group; n=678) over 2 years.
Results: Mean baseline characteristics were similar between groups. The average TI daily dose was 141.7±62.9 U. At 2 years, there was comparable reduction between groups in A1C (0.70% [TI], 0.59% [UC], p=0.30; see Figure). Total hypoglycemic event rates were 0.15 per patient-month in the TI group compared with 0.24 per patient-month for UC patients on insulin (p=0.03); mild/moderate (M/M), 0.15 per patient-month in the TI group compared with 0.24 per patient-month for UC patients on insulin (p=0.04); and severe, 0.53 per 100 patient-months in the TI group compared with 1.17 per 100 patient-months for UC patients on insulin (p=0.08). Weight gain in the TI group was +1.56 kg compared with 1.75 kg in the UC group (p=0.67).
Conclusion: In patients with T2DM, antihyperglycemic regimens containing prandial TI resulted in comparable A1C reductions, significant reductions in overall and M/M events, and a numerical reduction in severe hypoglycemic event rates.



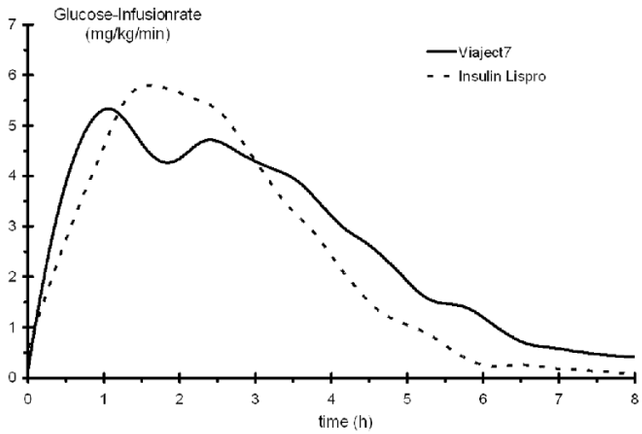
Supported by: MannKind Corporation

6

A novel pH-neutral formulation of the monomeric insulin VIAject® has a faster onset of action than insulin lispro

L. Nosek[1], T. Heise[1], F. Flacke[2], A. Krasner[2], P. Pichotta[2], L. Heinemann[1], S.S. Steiner[2];
[1]Profil Institut für Stoffwechselforschung GmbH, Neuss, Germany, [2]Biodel Inc, Danbury, USA.

Background and aims: VIAject® is a monomeric insulin with a very fast onset of action. Previous studies used a formulation of VIAject with a concentration of 25 U/ml and a pH of about 4 (VJ25). In this double-blind, cross-over glucose clamp study we compared the pharmacodynamic (PD) and pharmacokinetic (PK) properties of a novel formulation of VIAject with a concentration of 100 U/ml and a neutral pH (VJ7) with those of VJ25 and insulin lispro (LIS).
Materials and methods: Fourty-three people with type 1 diabetes (21 female, age 43 (21-65) years, BMI 24.1 (20-28) kg/m^2, HbA1c 7.5 (5.7-9.5)%) received 12 U of either insulin under euglycemic glucose clamp conditions (Biostator-clamp, duration 8h postdose).
Results: VJ7 was bioequivalent to VJ25 (90% confidence interval of the ratios for total insulin (INS) AUCs and INS_{max} within 0.80-1.25). Compared with LIS, VJ7 showed a significantly faster absorption (t- INS_{max} 23 vs. 60 min, difference -30 [90% Confidence Interval (CI) -35;-23] min; t-$INS_{50\%early}$ 8 vs. 22 min, difference -16 [CI -17;-14] min; p<0.05 respectively) and faster onset of action (time to early half-maximal glucose infusion rate (GIR) 25 vs. 44, difference -18 [CI -26;-10] min, p<0.05), a higher GIR-AUC in the first 60 min post-dose (176 vs 107, ratio 1.65 [CI 1.27;2.14] mg/kg, p<0.05) and a slightly higher value for AUC-GIR_{total} (1263 vs. 1095, ratio 1.15 [CI 1.06;1.26] mg/kg; p<0.05). GIR_{max} was similar between VJ7 and LIS (6.1 vs. 6.6, ratio 0.93 [CI 0.86;1.01] mg/kg/min) as was t-$INS_{50\%late}$ (136 vs. 129, difference 7 [CI -15;27] min) whereas the duration of action was longer with VJ7 (t-$GIR_{50\%late}$ 274 vs. 228, difference 50 [CI 25;73] min; p<0.05).
Conclusion: The novel, pH-neutral formulation of VIAject is bioequivalent to the previously used formulation and has a significantly faster absorption and onset of action than insulin lispro.



Supported by: Biodel Inc.

OP 2 Screening and prevention of gestational diabetes

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Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) Study: Frequency of gestational diabetes mellitus (GDM) at collaborating centers based on IADPSG consensus panel recommended criteria

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Background and aims: New criteria for GDM were recommended by the International Association of Diabetes in Pregnancy Study Groups (IADPSG). The aim is to apply the criteria to determine frequency of GDM at HAPO Study field centers.
Materials and methods: The IADPSG thresholds are one or more OGTT plasma glucose values => 5.1, 10.0, 8.5 mmol/l for fasting, 1-hr and 2-hr glucose respectively. These were applied to the blinded participants at the 15 field centers that collaborated in the HAPO Study.
Results: GDM was diagnosed in 16.1% of the blinded study population. An additional 1.7% was unblinded with an OGTT glucose value above pre-defined levels bringing the overall figure to 17.8%. However, there was considerable center-to-center variation of the study participants in maternal age, body mass index, frequencies of family history of diabetes and hypertension. Adjusting for these variables and for field center reduced, but did not eliminate center-center differences which in all likelihood reflect racial/ethnic group differences in the potential risk of disorders of glucose metabolism in these populations.
Conclusion: Using the diagnostic thresholds recommended by the IADPSG Consensus Panel, the frequencies of GDM show substantial variability between and within regions of the world. These variations may influence the future development of optimal, cost- effective strategies for detection and treatment of GDM.

Unadjusted Frequency of GDM at HAPO Field Centers

Field Center	Frequency (%)	Field Center	Frequency (%)
Bellflower, USA	22.9	Petah Tiqva, Israel	9.2
Chicago, USA	16.5	Beersheba, Israel	8.7
Providence, USA	14.2	Bangkok, Thailand	21.2
Cleveland, USA	23.7	Brisbane, Australia	12.1
Toronto, Canada	14.6	Newcastle, Australia	13.6
Belfast, UK	15.5	Singapore, Singapore	22.4
Manchester, UK	21.0	Hong Kong, China	**13.4**
Barbados, West Indies	9.9	HAPO Overall	16.1

Supported by: NIH, ADA

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Analysis of pregnancies after new IADPSG recommendation

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Background and aims: The IADPSG Consensus Panel recently has suggested new diagnostic criteria for gestational diabetes (GDM) diagnosis. Aim of our study was to evaluate clinical and metabolic characteristics and pregnancy outcome in women prior classified normal by Carpenter and Coustan criteria and now GDM according to the new recommendations.

Materials and methods: We retrospectively analyzed 3953 pregnancies: 2138 GDM and 1815 NGT by the new criteria: in the GDM group 112 women (2.8%) (GDM-NGT) were NGT with the old classification. We evaluated this group compared with GDM and NGT ones.
Results: As for clinical and metabolic parameter GDM-NGT women were younger (32.4 ± 4.5 yrs vs 33.4 ± 4.4 yrs, p=0.0039) and had a lower pre-pregnancy BMI (23.7 ± 4.3 kg/m^2 vs 24.7 ± 5.1 kg/m^2, p=0.005) than GDM ones, while gestational week at diagnosis and HbA1c levels at diagnosis and at the 3rd trimester were not different. The analysis of OGTT showed that ,at all point of curve, glucose levels were significant higher in GDM-NGT with respect to NGT ones (basal 90.5 ± 7.8 mg/dl vs 79.2 ± 6.8 mg/dl, p<0.0001; 60': 153.7 ± 18.8 vs 140.6 ± 23.7, p<0.0001, 120': 129.3 ± 20.6 vs 116.3 ± 20, p<0.0001). As for pregnancy outcome, caesarean section was 43.6% in GDM-NGT group, 41% in GDM (n.s.) and 31.1% in NGT (p=0.008); gestational age at delivery and birthweight were not different. Ponderal index (g/cm^3)was significant higher in GDM-NGT with respect to GDM and NGT (2.95 ± 0.61 vs 2.8 ± 0.41 and vs 2.77 ± 0.34, p<0.0001 respectively). A correlation analysis showed that pre-pregnancy BMI and basal glucose level were significant related with newborn ponderal index (p<0.0001, p<0.05 respectively).
Conclusion: So the new GDM diagnostic criteria recommended by IADPSG identified a new group of GDM women that, classified normal with Carpenter and Coustan criteria, show metabolic characteristics and pregnancy outcome similar to those of GDM women.

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New criteria for the diagnosis of gestational diabetes mellitus in comparison to former diagnostic criteria concerning maternal postpartum glucose levels and neonatal complications

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Background and aims: New criteria for the diagnosis of gestational diabetes (GDM) were recently proposed by the Consensus Panel of the International Association of Diabetes Pregnancy Study Groups (ICP). We aimed to examine whether these new criteria (GDM-ICP) select women and children at risk better than criteria of the Forth International Workshop Conference of GDM (GDM-WC4) using the 2-hour 75g oral glucose tolerance test (OGTT).
Materials and methods: This was a prospective longitudinal open study in five tertiary care centers in Austria. 1466 women underwent an 2-hour 75g-OGTT between the 24th and 28th gestational week and were treated if at least one value according to the GDM-WC4 was met or exceeded (GDM-WC4/1) in keeping with the recommendations of the Austrian and German Diabetes Association at that time. We evaluated the impact of risk factors, different thresholds (GDM-ICP vs. GDM-WC4/1) on fetal/neonatal complications and maternal postpartum glucose tolerance in pregnant women.
Results: Forty-nine percent of all women were diagnosed according to GDM-ICP, whereas forty-six percent according to GDM-WC4/1. GDM-ICP identified a higher rate of obstetrical complications. We found 6.1 % more large for gestational age neonates (p=0.0047), 3.26% more cesarean sections (p=0.0001) and 4.5% more neonates with birth weight >4000g (p=0.0047) than with the former criteria. The rate of women with risk factors (history of GDM or prediabetes, intrauterine fetal death, birth weight over 4000g, malformation, previous recurrent abortus, ethnic group at risk, adiposity, hypertension, dyslipidemia, preterm delivery, age, family history for DM2, glucosuria and macrosomia) detected by the GDM-ICP was increased by 2.6% (p<0.0001). However concerning impaired postpartum glucose tolerance we found no differences.
Conclusion: These results support the use of the new, more stringent criteria proposed by ICP for the diagnosis of GDM (GDM-ICP), because they can detect more obstetrical complications than the former diagnostic criteria (GDM-WC4/1) in a Central European population.
Supported by: Clinical research project award of the Austrian Diabetes Association

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ATLANTIC DIP: Prevalence and implications of abnormal glucose tolerance in pregnancy in Ireland

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Background and aims: While many authorities now recommend universal screening for gestational diabetes mellitus (GDM), this is not available in the Irish Health Service. Consequently, the true prevalence of abnormal glucose tolerance (and its implications) in pregnancy is unknown.
Materials and methods: ATLANTIC DIP (Diabetes In Pregnancy) is a multicentre study on the west coast of Ireland which offers universal screening for gestational diabetes mellitus (GDM) using a 75g oral glucose tolerance test at 24-28 weeks. Normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and GDM were defined according to standard WHO criteria. Maternal and foetal outcomes were recorded. Statistical analysis was performed using SPSS, and all results were adjusted for maternal age and body mass index (BMI).
Results: Between 2006 and 2008, 5,670 women (mean age 1.5±5.4yrs, mean BMI 26.9±5.1kg/m^2, 93.4% Caucasian) were screened. 5,100 (89.9%) women had a NGT, 431 (7.6%) had IGT, and 139 (2.5%) had GDM. The table below shows the Odds ratios [95% confidence intervals] of adverse maternal and foetal outcomes for IGT and GDM compared to NGT.
Conclusion: One in ten women screened had IGT or GDM. Given this prevalence and the associated significant adverse maternal and foetal outcomes, consideration should be given to universal screening for GDM throughout Ireland.

Adverse maternal and foetal outcomes

	IGT	GDM	IGT/GDM
Hypertensive disorder of pregnancy	1.4[0.9-2.1]	1.9[1.0-3.6]	1.5[1.0-2.2]
Polyhydramnios	3.9[1.4-10.6]	2.9[0.4-22.9]	3.7[1.4-9.5]
Instrumental delivery/ Caesarean Section(CS)	0.8[0.6-1.1]	3.0[1.6-5.6]	1.1[0.8-1.4]
CS	0.8[0.6-1.2]	3.8[2.1-6.9]	1.2[0.9-1.6]
Emergency CS	0.6[0.4-1.0]	4.5[2.3-8.6]	1.2[0.8-1.7]
Elective CS	1.1[0.7-1.6]	3.1[1.5-6.5]	1.5[1.1-2.1]
Congenital Malformations	2.4[1.0-5.6]	1.1[0.1-8.5]	2.1[1.0-4.7]
Small for gestational age	1.4[0.7-2.8]	3.8[1.5-9.4]	1.8[1.1-3.1]
Neonatal ICU admission	3.4[2.4-4.9]	9.5[5.3-16.9]	4.3[3.2-6.0]

Supported by: HRB

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Is maternal educational level a risk factor for gestational diabetes in caucasian women?

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Background and aims: Gestational Diabetes (GDM) is associated with features of Metabolic Syndrome (MS) and Cardiovascular Disease (CVD); low socio-economic status is a well known risk factor for these conditions. So, we performed this study to evaluate if maternal educational level (MEL) may be associated with the development of GDM as well as impaired glucose tolerance (IGT).
Materials and methods: From 2006 to 2009 we examined a cohort of Caucasian pregnant women attending 3-h 100-g OGTT in our Department of Diabetes. According to ADA criteria, GDM and IGT were diagnosed when almost 2 or 1 plasma glucose concentrations exceeded the cut-off levels, respectively. At the enrolment, upon collection of anthropometric and clinical parameters, we assessed the MEL on the basis of the level of attended school, evaluated by a questionnaire. So the women were categorized into three levels: low (primary school; L), intermediate (high school; I) and high (university; H) level.

Results: We studied 1012 Caucasian pregnant women (mean age 33.8±4.4 yrs;) at 27.4±4 wks of gestation: 20% L, 48.6% I and 31.4% H. L women were younger (33.4±6.2 vs 33.2 ± 4.3 and 34.9±3.7 yrs, p<0.0002) heavier (BMI 25.7±5.6 *vs* 24.2±5 and 23±3.9 kg/m^2, p<0.0001) with an higher rate of obesity (22.4% *vs* 12% and 6.9%, p<0.0001) and an higher weight gain during pregnancy(10.9±4.9 *vs* 10.4±5.8 and 9.7±3.6 kg, p<0.01) while H women were older than others (L 33.4±6.1 and I 33.2±4.3 *vs* H 34.9±3.7; p< 0.002). No differences were observed in family history for type 2 diabetes (27% L, 26% I and 21.5% H) and parity (primiparous: 45% L, 44% I and 40% H). IGT and/or GDM was diagnosed in 58(29%) L, 124(25%) I and 80(25 %) H women, without difference among the three groups. IGT and GDM diagnosis were not related to MEL, while after multivariate logistic regression analysis including all clinical and metabolic parameters, only prepregnancy BMI (F test value 12.148 p< 0.005) and age (F test value 9.318 p< 0.001) were independently associated with IGT or GDM diagnosis.
Conclusion: We failed to find any relationship between abnormal OGTT and maternal education levels. Probably in our population, differently than others, low education level is not a condition of deprivation and low health knowledge. Prepregnancy BMI and old age remain the best predictors of abnormal glucose tolerance during pregnancy. Obese and older women, independently of their education levels, have to be addressed to programs for preventing the development of glucose abnormalities during pregnancy.

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Metformin does not prevent gestational diabetes mellitus in women with polycystic ovarian syndrome
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Background: It is held that gestational diabetes mellitus (GDM) increases pregnancy complications and adverse pregnancy outcome. Metformin is also suggested to prevent pregnancy complications in women with polycystic ovary syndrome (PCOS). And some propose that metformin should be used to treat GDM.
Material and methods: In the first trimester of pregnancy, 273 PCOS women were randomized to metformin or placebo. At inclusion, gestational week 19 and 32, an OGTT were performed and pregnancy complications were recorded.
Results: At inclusion, 13 women in the placebo group and 10 in the metformin group had GDM. During the rest of the pregnancy 21/125 (16.9%) in the placebo group and 22/125 (17.6%) in the metformin group developed GDM (p=0.87). Insulin was required in four patients in the placebo group and none in the metformin group. We found no differences in mean birth weight (3524 ± 559 vs 3543 ± 601, p=0.82), in the incidence of preterm delivery (2/64 (3.1%) vs. 14/206 (6.8%), p=0.83) and preeclampsia (3/64 (4.7%) vs. 12/206 (5.8%), p= 0.69) between those who developed GDM and those who did not.
Conclusion: Our findings do not support the view that metformin prevents GDM in women with PCOS. This is rather surprising, given the effect of metformin in non-pregnant patients with diabetes mellitus type 2. In pregnant women insulin resistance increases. However, the pathogenetic mechanisms involved in the development of GDM may be different from the mechanism responsible for increased insulin resistance in non-pregnant women. Metformin may lack effect on the mechanism responsible in increasing gestational insulin resistance. The incidence of preterm delivery and preeclampsia were equal in PCOS women with and without GDM. This observation contradicts the view that GDM or blood glucose levels per se are directly involved in the development of preeclampsia and preterm delivery. If our observations are confirmed by future studies, other endocrine or metabolic aberrations associated with decreased glucose sensitivity must be responsible for the increased incidence of preeclampsia and preterm delivery seen in women with GDM. The observation that birth weight was not increased in offspring of PCOS women with GDM compared to those with normal glucose homeostasis is surprising. Our observations indicate that future studies on GDM should differentiate between PCOS women and non-PCOS women. In conclusion the present study does not support that metformin prevents the development of GDM in PCOS pregnancies. GDM in PCOS women is not associated with increased pregnancy complications or increased birth weight.
Supported by: Weifa AS

OP 3 Cardiovascular disease in type 1 diabetes

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Higher plasma advanced glycation endproducts levels are associated with incident cardiovascular disease and all-cause mortality in type 1 diabetes: a 12-year follow-up study
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Background and aims: Advanced glycation endproducts (AGEs) may constitute a pathophysiologic mechanism linking hyperglycaemia to the development of vascular complications. Therefore, we investigated the associations of plasma levels of AGEs with incident cardiovascular disease (CVD) and all-cause mortality in individuals with type 1 diabetes, and the extent to which any such associations could be explained by endothelial and renal dysfunction, low-grade inflammation and arterial stiffness.
Materials and methods: We prospectively followed 169 individuals with diabetic nephropathy and 170 individuals with persistent normoalbuminuria (205 men, mean age at baseline of 41±10 yrs) who were free of CVD at study entry and in whom levels of AGEs (expressed as the average of the z-scores of N$^{\varepsilon}$-(carboxymethyl)lysine, N$^{\varepsilon}$-(carboxyethyl)lysine and pentosidine) and other biomarkers were measured at baseline. The median follow-up duration was 12.3 (inter-quartile range: 7.6-12.5) years. Data were analysed with Cox regression analyses.
Results: During the course of follow-up, 82 individuals (24.2%) died; 85 (25.1%) suffered a fatal (n=48) and/or non-fatal (n=53) CVD event. The incidence of cardiovascular morbidity and mortality (Fig. 1A) and of all-cause mortality (Fig. 1B) increased with higher baseline levels of AGEs independently of traditional CVD risk factors (as detailed in footnote to figure): hazard ratio (HR)=1.30 (95%CI=1.02 to 1.65) and HR=1.29 (1.01 to 1.65) per 1 SD increase in AGEs score, respectively. Adjustment for estimated glomerular filtration rate (eGFR), but not for markers of endothelial dysfunction, low-grade inflammation and arterial stiffness, attenuated these associations to HR=1.16 (0.89 to 1.51) and HR=1.19 (0.90 to 1.57), respectively. Higher levels of AGEs were inversely associated with baseline eGFR: standardised regression coefficient=-0.29 (-0.38 to -0.20).
Conclusion: Higher levels of AGEs are associated with incident cardiovascular morbidity and mortality as well as all-cause mortality in individuals with type 1 diabetes. AGEs-associated renal dysfunction may partially explain these associations.

Table

	eGFR≥60; alb(-) n=497	eGFR<60; alb(-) n=93	eGFR≥60; alb(+) n=93	eGFR<60; alb(+) n=23	P-values eGFR	P-values urine albumin
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	P[#]	P[§]
Age (years)	60.5 (3.1)	61.5 (2.7)	60.8 (3.1)	60.5 (3.0)	0.004	0.749
Diabetes duration (years)	6.6 (6.1)	8.0 (6.9)	8.8 (5.5)	9.5 (5.2)	0.015	<0.001
BMI (kg/m^2)	29.9 (4.6)	31.0 (5.6)	30.7 (4.3)	31.4 (3.3)	0.025	0.105
Systolic BP (mmHg)	135 (16)	137 (14)	144 (19)	142 (13)	0.295	<0.001
Diastolic BP (mmHg)	79 (10)	81 (10)	82 (12)	61 (9)	0.480	0.049
HbA1c (%)	5.7 (1.0)	6.0 (1.1)	6.5 (1.2)	6.7 (1.7)	0.415	<0.001
PWV (m/s)	10.2 (2.0)	10.0 (1.8)	11.5 (2.5)	11.0 (1.9)	0.915	<0.001
LVMI (g/m^2)	118.5 (26.8)	119.0 (33.7)	131.5 (32.9)	146.9 (37.4)	0.327	<0.001
IMT (mm)	0.73 (0.18)	0.71 (0.18)	0.79 (0.17)	0.75 (0.26)	0.245	0.009

[#] Differences in means between patients with eGFR < 60 and ≥ 60 analysed with independent samples T test.
[§] Differences in means between patients with urine-albumin/creatinine ratio > 3 and ≤ 3 analysed with independent samples T test.

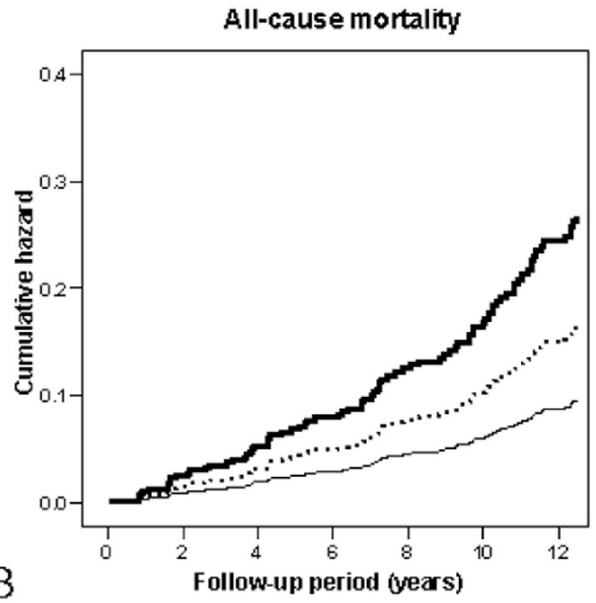

Figure 1. Cumulative hazard for CVD morbidity and mortality (A) as well as all-cause mortality (B) across tertiles of plasma AGEs score. Data are adjusted for age, sex, case-control status, duration of diabetes and HbA1c, mean arterial pressure, smoking status, total cholesterol, renin-angiotensin-aldosterone system, other antihypertensive agents, and con*tinuation ofmedication at baseline examination.*

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Repeated episodes of hypoglycaemia aggravate preclinical atherosclerosis in a group of young adult subjects with type 1 diabetes

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Background and aims: Type 1 Diabetes (T1D) is associated with an increased age-adjusted relative risk for atherosclerosis even though many of the classical cardiovascular risk factors (CVRF) are absent. While intensive insulin therapy reduces the risk of complications, it is associated with an increase in hypoglycaemia. Hypoglycaemia acutely provokes intense changes in haemodinamics and several haemorreological parameters. However, its role when chronically repeated as an accelerating factor for CV disease in diabetes is still far from proven.
Aims: To evaluate whether repeated episodes of hypoglycaemia represent an aggravating factor for vascular disease in subjects with T1D.
Materials and methods: Twenty-five T1D patients (34.6±7.8 years, 14 women, T1D duration 16.1±6.3 years, HbA_{1c} 6.6±1.0) with >4 non-severe-hypoglycaemia (NSH)/week and ≥2 severe hypoglycaemia (SH)/previous year and without additional CVRF, micro/macrovascular complications (including normal stress echocardiography) and no autonomic dysfunction were included (H-Group). In addition, 20 T1D subjects were selected as an age-sex matched control group presenting <2 NSH/week, no previous SH and without micro/macro/autonomic complications (C-Group). Previous to the CV evaluation, an acute induction of hypoglycaemia was performed to assess hypoglycaemia awareness. Inflammation/endothelial markers including leukocytes, Von Willebrand factor (VW), fibrinogen and ICAM-1 were also measured. B-mode-ultrasound was used to evaluate flow-mediated-dilatation in braquial artery (FMD) and intima-media-thickness (IMT) at both carotid/femoral sites. A 72-h blinded CGMS was recorded.
Results: As expected, H-Group subjects had significantly higher number of NSH/week and SH than C-Group (NSH/week 5.2±1.9 vs. 0.3±0.5 p<0.01). In addition, they displayed hypoglycaemia unawareness by Clarke's test and by symptomatic response to hypoglycaemia (Edinburgh's scale). Unsurprisingly, higher percentage of values and AUC <70mg/dl were observed in H-Group (14.2±8.9 vs. 6.3±7.1%, p<0.02 and 2.4±1.8 vs. 0.6±1.0 AUC, p<0.01). The percentage of maximal FMD was lower in H-Group than in C-Group (6.52±2.92 vs. 8.62±3.13%; p<0.05). A significantly higher IMT was observed at both carotid/femoral sites in H-Group in comparison to C-Group (carotid, 0.53±0.09 vs. 0.47±0.08mm; p<0.05 and femoral, 0.51±0.17 vs. 0.39±0.09mm; p<0.05). While in 10/25 subjects from the H-Group atherosclerotic plaques were detected this was not the case in any of the subjects from C-Group. Inflammation/endothelial dysfunction markers were higher in H-Group (leukocytes 7.0±1.8 vs. 5.6±1.4 $x10^3$/ul; VW 119±29 vs. 93±26 %; fibrinogen 2.82±0.64 vs. 2.29±0.44 g/L; ICAM-1 408±224 vs. 296±95 ng/ml; p<0.05 for all).
Conclusion: In addition to the induction of hypoglycaemia unawareness and an increased risk for severe hypoglycaemia, repeated hypoglycaemia in T1D is associated with a worse prognosis in terms of preclinical atherosclerosis profile. The precise mechanisms explaining this association remains to be clarified.
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Pulse pressure predicts all - cause and cardiovascular mortality but not deterioration in kidney function in type 1 diabetic patients

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Background and aims: Patients with type 1 diabetes have an elevated risk of early death due to cardiovascular disease (CVD) and development of end stage renal disease (ESRD). The aim of this analysis is to evaluate if pulse pressure (PP) (as an estimate of arterial stiffness) based on office arterial blood pressure predicts mortality, cardiovascular events and progression of diabetic nephropathy in patients with type 1 diabetes.
Materials and methods: A prospective observational follow-up study. The patients were followed for a median (range) of 8.2 (0.0-12.9) years. The cohort consisted of 898 type 1 diabetic patients. Of these, 456 patients had overt diabetic nephropathy (277 men; age (mean ± SD) 42.1 ± 10.5 years, duration of diabetes (mean ± SD) 28.3 ± 8.8 years, glomerular filtration rate (GFR) 76 ± 34 ml/min/1.73 m^2) and were followed with yearly measurement of GFR. The remaining 442 patients had longstanding type 1 diabetes and persistent normoalbuminuria (234 men; age 45.4 ± 11.5 years, duration of diabetes 27.8 ± 10.1 years).
Results: During follow-up 178 (19.8 %) patients died; 109 (12.1%) patients died from CVD causes and 99 (11.0%) patients developed ESRD. Individuals with elevated PP had significantly higher all-cause mortality (hazard ratio per 10 mmHg increase), (HR [95% CI] 1.18 (1.07 to 1.30); *p*=0.001, adjusted for sex, age, duration of diabetes, smoking, diastolic blood pressure, HbA_{1c}, eGFR, cholesterol, and history of CVD). For patients with diabetic nephropathy the adjusted HR for all-cause mortality was 1.16 (1.04 to 1.30) *p*=<0.001. Elevated PP also predicted CVD mortality (adjusted HR 1.27 (1.13 to 1.43) *p<0.001*), and non fatal CVD events (adjusted HR 1.12 (1.01 to 1.25) *p*=0.04). For fatal and non-fatal CVD combined, the adjusted HR was 1.13 (1.04 to 1.24) *p*=0.005). In patients with diabetic nephropathy, elevated PP was associated with higher risk of progression to ESRD (HR (per 10 mmHg increase) 1.27 (1.12 to 1.43) *p<0.001*) but this was not significant after adjustment (HR 1.03 (0.89 to 1.203) and elevated PP was not related to decline in GFR in patients with diabetic nephropathy.
Conclusion: Elevated office pulse pressure (arterial stiffness), predicts all-cause and CV mortality in type 1 diabetic patients. In contrast, office pulse pressure was not associated with progression of diabetic nephropathy.

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Decreased endothelial progenitor cells associated with poor glycaemic control predict both morphological microvascular and macrovascular impairment in type 1 diabetic children within three years

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Background and aims: The risk of cardiovascular death before the age of 40 is 20-fold increased in patients with type 1 diabetes mellitus (T1DM) com-

pared to those without. Vascular damage, which might be responsible for the increased risk, can be estimated by bone marrow derived Endothelial Progenitor Cells (EPC). EPC predict cardiovascular morbidity and mortality in patients without diabetes. Recently we have demonstrated that improvement or worsening of glycemic control resulted in indirect proportional changes in EPC in Type 1 diabetic children within one year. So, we asked whether a longer follow-up of these T1DM children would result in morphological changes of the macro- and microvasculature.
Materials and methods: We present data of 74 T1DM children: Gender: 51.4% female; Age: 12.7±0.3 years; Body Mass Index: 20.8±0.4; Glycated Haemoglobin A1c (HbA1c): 7.0-9.1 (range); diabetes duration 4.8±0.4 years (all at time of inclusion). Study visits were inclusion, after 1.0±0.1 and 2.7±0.2 years. EPC (flow cytometry) were measured at all visits.
For estimation of micro- and macrovascular damage two techniques were applied. For macrovascular damage - driven by plaque formation - Carotid Intima Media Thickness (IMT) was assessed by the average of 24 measurements of the vessel walls via high-resolution B-mode ultrasound. For microvascular damage - driven by vessel reactivity - tissue perfusion was investigated by Laser Doppler perfusion imaging (LDPI) and a post-occlusive reactive hyperemia provocation test. For statistical analysis a p-value < 0.05 was considered significant. Results are given in mean±STD or median (25;75 percentile).
Results: EPC at inclusion: 3410/106WBC (2515;4237), EPC at one year: 3334/106WBC (2929;3898). LDPI parameters after 2.7 years: time to absolute peak 9.5s (6.0;21.3), time to baseline: 80.5s (63.0;108.3), Total Time (LDPI-TT): 95.5s (78.0;118.3), Perfusion at absolute peak 1.435 (1.020;2.050), Perfusion at baseline: 0.390 (0.258;0.598), Difference of perfusion at peak and baseline: 1.055 (0.610;1.513). IMT at latest follow-up was 0.285 (0.258;0.350). EPC at earlier time points were significantly predictive for LDPI-TT (R=-0.259; p=0.042) and IMT (R=-0.344; p=0.003). Cross-sectional the following parameters were associated with LDPI-TT: HbA1c (R=0.255; p=0.045); mean-blood-glucose (R=0.331; p=0.013) and aspartate aminotransferase (ASAT) (R=0.315; p=0.015). Associations with IMT were systolic blood pressure (R=0.248; p=0.035); ASAT (R=0.332; p=0.005); alanine aminotransferase (R=0.280; p=0.019); duration of diabetes (R=0.0255; p=0.028).
Conclusion: This is the first study demonstrating an association of EPC with disturbed micro- and macrovascular function in children with Type 1 Diabetes mellitus after a short observation period. Thus, it is most likely that glucose fluctuation with high HbA1c lead from depression of bone-marrow derived EPC to microvascular changes (LDPI), to plaque formation (IMT) and finally to premature cardiovascular disease and death.

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Early signs of atherosclerosis in diabetic children on intensive insulin treatment: a population based study
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Objective: Young adults with type 1 diabetes are at increased risk of early asymptomatic atherosclerosis and cardiovascular morbidity and mortality are substantially increased in this group of patients. The aim of this study was to evaluate early stages of atherosclerosis and predisposing factors in childhood diabetes compared to age- and sex matched healthy control subjects.
Research design and methods: All children and adolescents with type 1 diabetes, aged 8-18 years in Health Region South-East in Norway were invited to participate in the study (n=800). 40% (n=314) agreed to participate and were compared to118 age-matched healthy controls. Carotid artery Intima Media Thickness (cIMT) and elasticity was measured using standardized methods.
Results: Mean age of the diabetic patients was 13.7 years, diabetes duration 5.5 years and HbA1c 8.4%. 97% were using intensive insulin treatment, 60% insulin pumps. Diabetic patients had more frequently elevated cIMT than healthy controls: 19.5% were above 90th centile of normal healthy controls and 13.1% above 95th centile (p<0.001). Mean cIMT was higher in diabetic boys compared to healthy controls (0.46mm /SD 0.06mm vs. 0.44mm/SD 0.05mm, p=0.04) but not significantly so in girls. There was no significant difference between the groups regarding carotid distensibility, compliance and wall stress. None of the subjects had atherosclerotic plaque formation. Although within the normal range the mean values of systolic blood pressure (SBP), total cholesterol, LDL-cholesterol and ApoB were significantly higher in the diabetic patients than in healthy controls.
Conclusion: Despite short disease duration, intensive insulin treatment, fair glycemic control and no signs of microvascular complications, children and adolescents with type 1 diabetes had slightly increased cIMT compared to healthy controls, the differences being more prominent in the boys.
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Colesevelam effects on LDL-C, HbA_{1c}, and GLP-1 in type 1 diabetes mellitus (T1DM)
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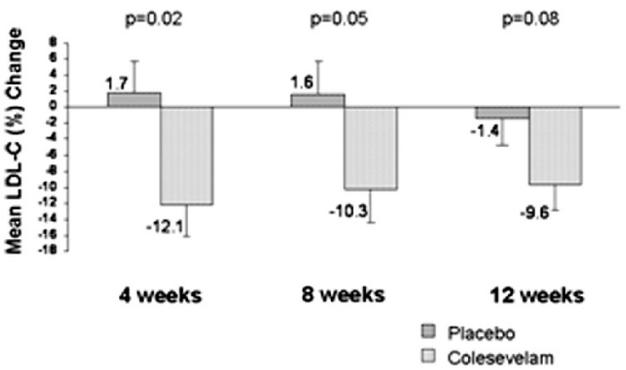
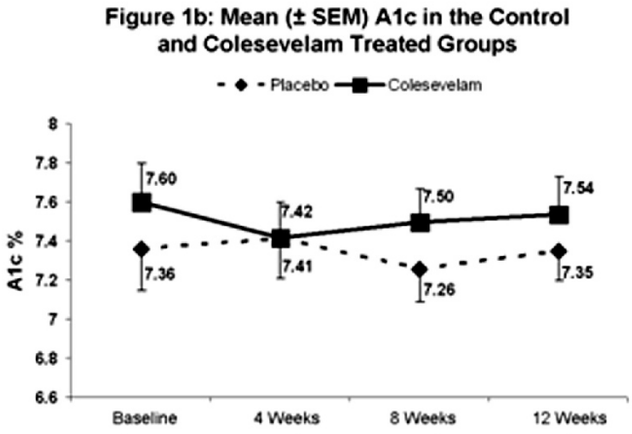
Background and aims: Colesevelam is indicated to lower LDL-C in hyperlipidemia and improve glycemic control in type 2 diabetes (T2DM). The clinical effects in subjects with T1DM have not previously been evaluated.
Materials and methods: This was a double-blind, randomized, investigator-initiated, single center, 12-week pilot study that evaluated 40 adults (36.4±9.4 yr) with T1DM and hyperlipidemia. The study was powered to demonstrate a treatment difference of >10% LDL-C reduction from screening. Subjects were randomized to receive either 3.75 g/day colesevelam (n=20) or placebo (n=20) for 12 weeks. LDL-C and A1c levels were assessed at screening (week -2), baseline (week 0) and every 4 weeks for the duration of the study. All subjects had a 4-hour Boost Plus® meal-challenge test (MCT) at baseline (week 0) and at the end of study (week 12). Study drug was administered at time 0 of the MCT. Incretin levels including GIP and GLP-1 were assessed during the MCT at the following time points (-30, 0, 30, 60, 120, 180 and 240 min). Screening demographics were similar in both treatment groups. Thirty-six subjects (n=18 in each group) completed the study. Compliance for both treatment groups was >88%.
Results: All data presented are from the intent-to-treat population with last observation carried forward. Colesevelam treatment resulted in a significant difference in the least squared mean percent change in LDL-C at 4 weeks (1.7% vs. -12.1%, difference between means 13.84 [95%CI: 2.2, 25.5], p=0.02), and this difference was marginally significant at 8 (p=0.05) and 12 weeks (p=0.08) (Figure 1a). Treatment also resulted in a statistically significant change in A1c from screening at week 4 (-0.19, p=0.04), however this did not remain significant for the study duration (Figure 1b).
Colesevelam resulted in significant median increases in GLP-1 levels during the first 2 hours of the baseline MCT compared to placebo following the first dose of study drug. There was no difference in GLP-1 levels between groups at the week 12 MCT. The GIP levels were similar between the two groups at baseline and 12 week MCTs.
There were no significant differences between or within groups for fasting glucagon, fasting and postprandial glucose, insulin dose, weight or BMI.
Conclusion: Results from this small pilot study suggest that Colesevelam treatment lowers LDL-C in patients with TIDM. While improvements in A1c were observed at week 4, significance was not maintained for the study duration. This effect might be explained by sample size, study duration, and/or chance. The treatment group showed an increase in GLP-1 levels at baseline, which may explain their improvements in A1c at week 4 of this study. However, the effect of Colesevelam on glycemic control in subjects with T1DM requires further clinical study involving a larger number of subjects over a longer duration.

Figure 1a: Mean (± SEM) Percent Change in LDL-C from Baseline (Week 0) (LOCF analysis)





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OP 4 Gastrointestinal factors and insulin secretion

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Obestatin and ghrelin bind to human pancreatic islet endothelial cells and inhibit apoptosis in high glucose condition

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Background and aims: Pancreatic islet microendothelium exhibits unique structural and functional features, in an interdependent physical and functional relationship with the beta cells. Glucose toxicity is not solely restricted to beta cells, but affects also survival of pancreatic islet endothelial cells, thus contributing to beta cell function impairment and cell loss. Studies indicate that gastrointestinal products of the ghrelin gene, obestatin (Ob), acylated ghrelin (AG) and its major circulating form, unacylated ghrelin (UAG) stimulate proliferation and prevent apoptosis of pancreatic β cells and human islets. We aimed to investigate whether these peptides would display survival effects also in human pancreatic islet microendothelial cells (MECs) cultured in high glucose conditions.
Materials and methods: Islet MECs were cultured in 28 mmol/L glucose concentration and, in parallel cultures, stimulated with AG, UAG or Ob (10 nM). Apoptosis was assessed by photometric enzyme immunoassay measuring mono- and oligonucleosomes as an index of DNA fragmentation, by Hoechst staining of apoptotic cells, and by Caspase 3 activity. Western-blot analyses for P-Akt/Akt, P-ERK/ERK pathways and for Bcl-2 (anti-apoptotic gene) and Bax (pro-apoptotic gene) were performed. Blockade of PI3K/adenylyl cyclase/cAMP/protein kinase A signalling was also performed.
Results: Islet MECs express the AG receptor (GRLN-receptor), assessed by RT-PCR analysis, and bind Ob, as assessed by immunofluorescence. In high glucose condition, AG, UAG and Ob inhibited islet MEC apoptosis, activating PI3K/Akt and ERK1/2 phosphorylation and upregulating intracellular cAMP. Further, Bcl-2 expression increased and Bax expression decreased. Blockade experiments counteracted the anti-apoptotic effects.
Conclusions: These data provide evidence that the ghrelin gene-derived peptides bind to, and promote survival of, islet microendothelial cells. The anti-apoptotic effects involve the PI3K/Akt, ERK1/2 and cAMP/PKA pathways. These peptides could therefore represent a potential tool to improve islet vascularization and, indirectly, islet function.
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Improved glucose-lowering, insulin-releasing and anorexigenic actions of a novel chemically modified analogue of oxyntomodulin, (D-Ser^2)Oxm[mPEG-PAL]

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Background and aims: Oxyntomodulin (Oxm) is a 37 amino acid peptide hormone released from intestinal L-cells in response to feeding. Oxm has been shown to exhibit a range of potentially beneficial actions for the alleviation of obesity-diabetes. However, possible exploitation of Oxm-based therapies has been severely restricted due to proteolytic degradation of the peptide by the ubiquitous enzyme dipeptidylpeptidase-IV (DPP-IV). Therefore, the aim of this study was to assess the glucose-lowering, insulin-releasing and anorexigenic actions of novel chemically modified, enzyme resistant analogues of Oxm.
Materials and methods: Oxm, (D-Ser^2)Oxm and (D-Ser^2)Oxm[mPEG-PAL] (all >97% purity) were incubated (0, 2, 4, 8 and 24 h) with DPP-IV (5 mU; n=3) to assess enzyme stability and with clonal pancreatic BRIN-BD11 cells to evaluate acute (20 min; n=8) insulin secretion. Cyclic AMP production (n=4) was examined using GLP-1 and glucagon receptor transfected cells. *In vivo* effect of Oxm analogues on glucose homeostasis, insulin secretion, food intake and body weight were examined in obese diabetic *(ob/ob)* mice.
Results: (D-Ser^2)Oxm[mPEG-PAL] displayed enhanced DPP-IV resistance compared to (D-Ser^2)Oxm and Oxm (13.4 and 1.6-fold, respectively; P<0.001). Oxm, (D-Ser^2)Oxm and (D-Ser^2)Oxm[mPEG-PAL] stimulated cyclic AMP production in a concentration-dependent manner with similar potency in BRIN-BD11 cells with EC_{50} values of 1.84 ± 0.09 nmol/l; 1.81 ± 0.37 nmol/l; and 1.97 ± 1.21 nmol/l, respectively. This was associated with

dual action at GLP-1 and glucagon receptors, although EC_{50} values were 15 to 200-fold greater than respective natural ligands. All peptides significantly stimulated insulin secretion (1.6 to 2.5-fold; P<0.001) in a concentration-dependent and equipotent manner. Acute administration (25 nmol/kg bodyweight) of (D-Ser^2)Oxm[mPEG-PAL] and (D-Ser^2)Oxm reduced plasma glucose (40-50% reduction; P<0.01) and food intake (1.1 to 1.4-fold; P<0.05 to P<0.01), while plasma insulin levels were elevated (1.5 to 1.8-fold; P<0.01 to P<0.001) in *ob/ob* mice. Animals treated with (D-Ser^2)Oxm[mPEG-PAL] and (D-Ser^2)Oxm four or eight hours prior to a glucose load (18 mmol/kg bodyweight) had significantly decreased plasma glucose concentrations (1.2 to 2.0-fold reduction; P<0.05 to P<0.001) compared to Oxm-treated animals. Once-daily administration of (D-Ser^2)Oxm[mPEG-PAL] for 14 days to *ob/ob* mice decreased food intake (1.2 to 1.5-fold; P<0.05 to P<0.001), body weight (1.1 to 1.5-fold; P<0.05 to P<0.01), plasma glucose (30-50%; P<0.05 to P<0.01) and increased plasma insulin (1.4 to 1.5-fold; P<0.01). Furthermore, daily (D-Ser^2)Oxm[mPEG-PAL] administration improved glucose tolerance (1.2 to 1.6-fold; P<0.05 to P<0.001), increased glucose-mediated insulin secretion (1.5 to 1.8-fold; P< 0.05 to P<0.01), pancreatic insulin content (1.5-fold; P<0.001), circulating adiponectin (1.4-fold; P<0.01) and decreased both visfatin (1.3-fold; P<0.01) and triglyceride levels (1.4-fold; P<0.01).
Conclusion: The ability of enzyme resistant (D-Ser^2)Oxm[mPEG-PAL] to improve glucose homeostasis, insulin secretion, satiety, body weight and markers of fat metabolism suggests significant promise for Oxm-based therapies for obesity-diabetes.

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Synaptotagmin-7 is a positive regulator of glucose-induced GLP-1 secretion

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Background and aims: Glucagon-like peptide 1 (GLP-1) is produced by highly specialized gut endocrine L-cells. Despite the demonstrated medical benefits of targeting GLP-1 in the treatment of diabetes, very little is known about the molecular control of GLP-1 secretion. Similar to other endocrine cells, such as islet β-cells, L-cells are electrically excitable, and express ATP-sensitive K-channels and calcium channels. Furthermore, GLP-1 is stored in secretory granules, and GLP-1 granule exocytosis is triggered by increased intracellular calcium levels resulting from stimulation by nutrient, neural and paracrine factors. Although the calcium dependence of GLP-1 granule exocytosis is well established, the identities of calcium-sensing proteins in GLP-1 exocytosis remain elusive. Several members of the synaptotagmin family have been identified as calcium sensors in neurotransmitter and hormone release. In particular, synaptotagmin-7 regulates insulin and glucagon secretion in pancreatic islets, and catecholamine release in chromaffin cells. Considering the functional importance of synaptotagmin-7 in endocrine and neuroendocrine cells, we tested the involvement of synaptotagmin-7 in the regulation of GLP-1 secretion.
Materials and methods: Synaptotagmin-7 expression in intestinal L-cells was tested by immunohistostaining of frozen mouse intestinal sections for synaptotagmin-7 and GLP-1. GLP-1 levels were measured in synaptotagmin-7 knockout mice challenged by glucose ingestion. Glucose-stimulated GLP-1 release was also tested in lentiviral-mediated synaptotagmin-7 knockdown GLUTag cells using cell secretion assay and membrane capacitance measurements. Data are presented as mean ± SEM.
Results: Synaptotagmin-7 was present in L-cells, as demonstrated by its co-localization with GLP-1. Synaptotagmin-7 deletion *in vivo* resulted in a reduction of GLP-1 secretion (683 ± 48 and 360 ± 40 pmol/l/h, control and synaptotagmin-7 knockout, respectively, p < 0.001). Synaptotagmin-7 knockdown GLUTag cells showed a ~50% decrease in glucose-stimulated GLP-1 secretion as determined by cell secretion assay and by membrane capacitance measurements (24 ± 4 and 12 ± 3 fF s^{-1}, control and synaptotagmin-7 KD, respectively, p < 0.05). Synaptotagmin-7 knockdown cells exhibited normal calcium responses, indicating the secretion defects occurred at or downstream of the calcium sensing step.
Conclusion: Taken together, our results demonstrate the importance of synaptotagmin-7 in the regulation of GLP-1 secretion, consistent with its proposed role as a calcium sensor in endocrine and neuroendocrine secretion.
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Assessment of the metabolic effects of the gut peptide xenin on insulin secretion, glycaemic control and satiety

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Background and aims: Recently significant focus has been directed towards the role of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) in the aetiology and treatment of type 2 diabetes. However, peptides co-secreted from the same enteroendocrine cells are less well studied. The present study examines the *in vitro* and *in vivo* metabolic effects of xenin, a peptide co-secreted with GIP from intestinal K-cells.
Materials and methods: Cyclic-AMP production (n=4) and insulin releasing activity (n=8) of xenin was measured in acute (20 min) studies with clonal pancreatic BRIN-BD11 cells. Effects of xenin on membrane potential and intracellular Ca^{2+} were also examined in BRIN-BD11 cells. The glucagon-secreting alpha cell-line, αTC1.9 was used to assess glucagon releasing activity of xenin (n=8). Effects of xenin on plasma glucose and insulin concentrations were examined in overnight fasted normal mice (n=8; 16-20 weeks of age) following subcutaneous (s.c.) injection (25 nmol/kg bodyweight) in combination with glucose (18 nmol/kg bodyweight). To assess duration of biological action, groups of mice were injected with xenin (25 nmol/kg) 30 or 60 min prior to glucose load. For food intake studies, mice were fasted for 12 hours prior to s.c. injection of 50, 100 or 500 nmol/kg xenin. Mice were then allowed free access to normal chow and cumulative food intake monitored.
Results: In clonal BRIN-BD11 cells xenin (10^{-6} M) stimulated insulin secretion at 5.6 (P<0.05) and 16.7 (P<0.01) mmol/l glucose levels compared to respective controls. Xenin also exerted an additive effect (P<0.05 to P<0.01) on GIP and GLP-1 mediated insulin secretion. Xenin did not stimulate cellular cyclic AMP production, alter membrane potential or elevate intracellular Ca^{2+} concentrations. Similarly, *in vitro* glucagon release was not affected by xenin. In normal mice, administration of xenin together with glucose significantly (P<0.05 to P<0.001) reduced individual glucose levels compared to glucose control. Moreover, the area under the curve (AUC) for glucose was significantly lower after administration of xenin compared with glucose administered alone (2.3-fold; P<0.001). This was associated with a significantly enhanced overall glucose-mediated insulin secretory response for xenin compared to glucose alone (1.4-fold; P<0.05). Administration of xenin 30 min previously significantly (P<0.05) decreased individual glucose levels 60 minutes post s.c. glucose injection in normal mice. This was associated with substantially enhanced (2.1-fold; P<0.05) overall insulin release compared to respective controls. Administration 60 min prior to glucose challenge annulled the glucose lowering and insulin releasing effects of xenin. Assessment of effects of xenin on feeding revealed that xenin did not induce a significant change in cumulative food intake in overnight fasted mice when administered at a dose of 50 or 100 nmol/kg. However, when mice received a dose of 500 nmol/kg, xenin induced a 60% decrease (P<0.01) in food intake 30 minutes post injection.
Conclusion: These data indicate that xenin is a short acting peptide with potentially important metabolic effects on blood glucose control. Generation of specific xenin antagonists or longer acting xenin mimetics may be useful in assessing the overall biological consequence of xenin-mediated actions.

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The effects of brain glucagon-like peptide-1 on peripheral glucose homeostasis

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Background and aims: Glucagon-like peptide-1 (GLP-1) is a gut peptide that promotes glucose homeostasis through regulation of islet-cell hormone secretion, gastric emptying, and hepatic function. GLP-1 is also synthesized in the brain, and recent findings indicate that central GLP-1 plays a role in the regulation of peripheral glucose homeostasis through effects on insulin secretion, hepatic glucose production, and insulin sensitivity. To gain further insights into those processes, we investigated the effects of central GLP-1 receptor (GLP-1r) activation and antagonism on insulin and glucagon release before and during experimental hyperglycemia in rats (*study I*). In addition, we studied the effects of central GLP-1r antagonism on glucose tolerance after meal ingestion in rats (*study II*).

Methods and materials: In *study I*, 30 male Long Evans rats were equipped with third cerebral ventricular (i.c.v.) cannulae and catheters in the carotid artery and jugular vein. After an overnight fast freely-moving animals had continuous i.c.v. infusion with GLP-1 (18 μg/hr), the GLP-1r antagonist Exendin 9-39 (Ex-9) (100 μg/hr), or saline for 125 min; all rats received 8 μl/hr of infusate. Sixty min after the start of i.c.v. infusion, blood glucose was raised to 230 mg/dl with a primed i.v. infusion of 25% glucose. For 65 min the glucose infusion rate was varied to maintain hyperglycemia (230 mg/dl). Arterial blood was sampled during the infusion period every 5-15 min for measurements of blood glucose and plasma levels of insulin, glucagon, and corticosterone. In *study II*, 22 overnight fasted and freely-moving rats with i.c.v. cannulae and i.a. catheters were infused i.c.v. with Ex-9 (100 μg/hr), or saline for 150 min. Thirty min after the start of i.c.v. infusion bottles of Ensure liquid diet (5.5 ml) were made available for 10 min. Because the rats were trained to eat Ensure, they voluntarily consumed the meal within 5 min. After meal ingestion, arterial blood was sampled every 15 min for up to 120 min for measurements of blood glucose.
Results: In *study I*, infusion of i.c.v. GLP-1 increased fasting blood glucose levels (105.5 ± 3.8 vs 91.5 ± 2.9 mg/dl), reduced the average glucose infusion rate required to maintain the glucose clamp (40.0 ± 1.5 vs 54.3 ± 2.8 mg/kg/min), and significantly reduced plasma insulin levels (AUC 14667 ± 1210 vs 20168 ± 1883 pM·min) compared to the saline group ($p < 0.05$ for each). GLP-1-treated rats also had significantly higher glucagon and corticosterone levels ($p < 0.05$ for each) than Ex-9- and saline-treated rats. Surprisingly, there were trends for i.c.v. Ex-9 to reduce insulin levels ($p = 0.0641$) and raise plasma glucagon ($p = 0.1009$). In *study II*, i.c.v. Ex-9 increased blood glucose levels after meal ingestion relative to the saline group, and the area under the curves (4844 ± 233 vs 4150 ± 188 (mg/dl)·min) were significantly higher ($p < 0.05$) in the Ex-9-treated rats compared to the saline group.
Conclusion: These results show that central GLP-1r activation has multiple effects on glucose homeostasis in rats. In the doses used in this study GLP-1 activated the stress response, likely via autonomic-induced suppression and stimulation of insulin and glucagon secretion, respectively. Central GLP-1r blockade using Ex-9 was associated with lower insulin secretion, higher glucagon levels, and higher glucose levels after meal ingestion, supporting a role for brain GLP-1 in the regulation of islet function and peripheral glucose homeostasis.
Supported by: NIHDK

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TGR5-mediated GLP-1 secretion
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Background and aims: The incretin hormone, GLP-1, is secreted from intestinal L-cells in response to food ingestion, however little is known about how L-cells detect luminal nutrients. Bile acids are also released into the gut lumen in response to nutrients and have been associated with glucose homeostasis and GLP-1 secretion. The aim of this study was to investigate the expression of the bile acid sensitive G-protein coupled TGR5 receptor in primary L-cells and its role in GLP-1 secretion.
Materials and methods: mRNA expression was analyzed by qRT-PCR in L-cells purified from transgenic mice with fluorescently labeled proglucagon-expressing cells. GLP-1 secretion was assayed in primary colonic cultures and GLUTag cells. FRET based $[cAMP]_i$ and ratiometric $[Ca^{2+}]_i$ imaging experiments were performed on GLUTag cells.
Results: TGR5 mRNA expression is highly enriched in L-cells compared to control intestinal epithelial cells. The bile acids deoxycholic acid, lithocholic acid and taurolithocholic acid (TLCA) and a TGR5 agonist increased GLP-1 secretion and enhanced the glucose-triggered response. The GLP-1 response to TLCA and the TGR5 agonist from GLUTag cells was attenuated with TGR5 siRNA treatment. Consistent with signaling via G_s coupled pathways, the TGR5 agonist and TLCA elevated $[cAMP]_i$ in GLUTag cells. The TGR5 agonist triggered $[Ca^{2+}]_i$ and enhanced glucose-triggered $[Ca^{2+}]_i$ responses, consistent with previously observed responses of GLUTag cells to elevated cAMP.
Conclusion: Primary L-cells express the bile acid sensitive TGR5 receptor, which may contribute to the incretin response via the elevation of $[cAMP]_i$ and $[Ca^{2+}]_i$. The synergistic stimulation of GLP-1 release may be possible by combined activation of GPCRs and glucose-sensing pathways.
Supported by: MRC, WT

OP 5 Somatic and autonomic neuropathy

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Near-normoglycaemia prevents the development of polyneuropathy, a 24-year prospective study from the diagnosis of type 1 diabetes
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Background and aims: Whether near-normoglycaemia instituted from the diagnosis of type 1 diabetes onward may completely or partially prevent the development of polyneuropathy and autonomic neuropathy has not been previously determined. To address this issue we conducted a long-term prospective study.
Materials and methods: We examined 32 newly diagnosed Type 1 diabetic patients (21 male, 11 female) aged 12-36 (mean±SEM: 20.3±1.0) years who were followed over 24 years. Motor and sensory nerve conduction velocity (MNCV, SNCV), vibration perception threshold (VPT), thermal detection thresholds (TDT), coefficient of R-R interval variation at rest (CV), and clinical assessment were performed at the time of diagnosis, after 3 and 12 months, and thereafter biennially for up to 24 years.
Results: During the 24-year period, 11 patients had mean HbA1c levels <7.0% according to the current guidelines (6.5±0.1%; Group 1), whereas 21 patients had mean HbAc levels ≥7.0% (8.3±0.2%; Group 2). Within the first 4 years, reduced sensory NCV and cardiac autonomic function, rather than elevated VPT and TDT were observed as markers indicating initial nerve dysfunction in Group 2 as compared with Group 1. After 24 years, MNCV was significantly faster in Group 1 than in Group 2 in the median (55.5±1.6 vs 48.9±1.6 m/s), ulnar (56.5±1.5 vs 49.3±1.7 m/s), and peroneal nerve (44.7±1.6 vs 36.8±2.5 m/s) and SNCV was faster in the median (53.6±1.6 vs 45.5±2.8 m/s), ulnar (54.7±1.8 vs 43.0±3.9 m/s), and sural nerve (44.5±1.8 vs 35.5±2.6 m/s) (all $p<0.05$). In Group 1 the decline in peroneal MNCV and sural SNCV was -0.1 and -0.2 m/s/year, respectively, comparable to a non-diabetic control group (n=15). In contrast, Group 2 showed corresponding losses of -0.4 and -0.5 m/s/year, respectively. Impairment in VPT, TDT, and CV also developed at a significantly faster rate in Group 2 than in Group 1 (all $p<0.05$). After 24 years, none of the patients in Group 1, but 12 (57%) of those in Group 2 developed clinical polyneuropathy confirmed by NCV. Two patients in Group 2 died during the period studied.
Conclusion: Near-normoglycaemia maintained from the diagnosis of type 1 diabetes over the next 24 years effectively prevented the development of clinical polyneuropathy, a 3-fold faster annual hyperglycaemia-induced decline in NCV, and deterioration in sensory and cardiac autonomic function, whereas poor glycemic control constituted the paramount permissive factor contributing to the evolution and progression of neuropathy in the majority of type 1 diabetic subjects.

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A double-blind randomised study comparing the effects of pregabalin, duloxetine and amitryptiline on aspects of neuropathic pain, mood and sleep in diabetic subjects with painful neuropathy
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Background and aims: The mechanisms of painful diabetic neuropathy are poorly understood and drug treatment if often unpredictable, with both an overall disappointing response rate, and generally poor patient tolerance to long term use. The main aim of the study was to try and identify which commonly used agent may have most beneficial effect on both pain and quality of life.
Materials and methods: Subjects with neuropathic pain (Leeds Assessment of Neuropathic Symptoms and Signs (LANSS)>12) were recruited from 2 hospital diabetes clinics. Subjects were required to have a score >25 on the Mini Mental State Exam (MMSE). The 36 day study (placebo day 1-8) had a double blind, parallel groups design with three treatment arms consisting of either amitriptyline (50mg followed by 75mg), pregabalin (300mg followed by 600mg) or duloxetine (60mg followed by 120mg). Subjects previously on any of neuropathic medications had an appropriate wash out period prior

to study entry, and all other analgesic medications were withdrawn where possible. Subjects were studied on days 6-8, 20-22, and 34-36. 65 of 83 (78%) randomised subjects (mean age 65 (38-83) years, 69% male), completed the study. Subjects completed daily diaries for pain (brief pain inventory (BPI) and the Short-form McGill visual analogue scale (VAS).
Results: In all three groups there was a significant decrease ($p < 0.05$) in pain severity (BPI severity and VAS) over time but there was no difference between the three treatments in reducing pain severity. Decrease in pain severity was significant in all treatment arms from placebo to low dose ($p<0.05$ for pregabalin and amitriptyline, $p<0.01$ for duloxetine), but no significant additional treatment benefit was seen in any group with increased drug dosage. Patients on duloxetine showed a significant decrease in pain interference over time, both from placebo to low dose, and from low dose to high dose ($p < 0.05$), and an improvement in BPI mood (<0.05). All three study drugs doses improved pain interference on sleep as assessed by the BPI ($p<0.05$) with no significant difference between any of the treatments.
Conclusion: Deciding on the ideal treatment drug for painful diabetic neuropathy remains an enigma, with seemingly little difference between conventional drugs on pain outcomes. Duloxetine, amitryptiline and pregabalin all improved subjective pain as assessed by the BPI and VAS and pain interference on sleep with no significant difference between any of the treatments. With duloxetine there was also a reduction in pain interference and mood.
Supported by: PF

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New insights into central pain processing in painful diabetic neuropathy: a functional magnetic resonance imaging study
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The exact pathophysiology underlying painful-diabetic peripheral neuropathy (DPN) remains unknown although several peripheral and central mechanisms have been postulated. An investigation of the brain's responses to acute painful stimuli may provide insights into the impact of the neuropathic process on nociceptive processing, thereby identifying key areas possibly affected by DPN.
Methods: 6 subjects with Painful-DPN underwent detailed neurophysiological assessments and functional magnetic resonance imaging (fMRI). All subjects had severe neuropathic pain below the knees. Before fMRI, heat pain was applied to the anterior thigh (control region) and dorsum of the foot to establish the level of noxious thermal stimulus which elicited a response of ≥7/11 point Likert scale. This was repeated inside the scanner alternating between pain-free baseline and noxious stimulus in a box-car design. Images were analysed using statistical parametric mapping SPM5. Following spatial preprocessing, first-level functional images were produced comparing baseline and heat pain states. Images were combined at the group level in a random effects model.
Results: Painful stimuli delivered to the neuropathic foot site were associated with significantly *reduced* activation in the left posterior insular cortex compared with painful stimuli administered to the thigh (stereotactic coordinates: x=-38, y=-20, z=16; peak t[5]=12.57; 55 voxels exceeded height threshold $p<0.01$, uncorrected). Conversely, compared with painful stimuli delivered to the thigh, painful stimuli administered to the neuropathic foot site evoked significantly *greater* brain activation in the right medial prefrontal cortex (x=12, y=36, z=34; t[5]=9.11; 42 supra-threshold voxels).
Discussion: The posterior insular cortex serves as an interface between afferent processing mechanisms and more cognitively orientated mechanisms. Relative lack of activation in this region during acute noxious thermal stimulation of the foot suggests a complex alteration of the pain experience in Painful-DPN. Prefrontal cortex is associated with high-order cognitive and emotional functions. Increased activation in this region during noxious stimulation of the foot suggests greater emotional/cognitive involvement of acute pain processing in Painful-DPN. These preliminary results suggest that painful stimuli delivered to neuropathic and symptom-free sites in DPN evoke differential activation of distinct cortical regions, which could reflect abnormal central pain processing.

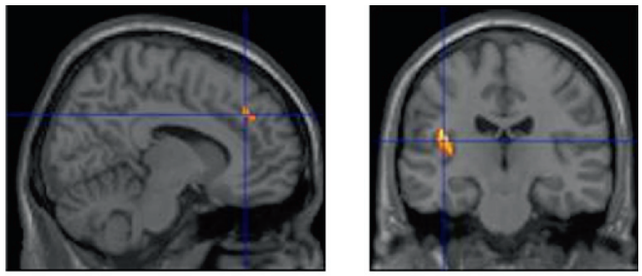

Figure 1: Greater brain activation foot vs. thigh right medial prefrontal cortex (a) and thigh vs. foot left posterior insular cortex (b).

Supported by: JDRF

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Obstructive sleep apnoea is independently associated with peripheral neuropathy in patients with type 2 diabetes
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Background: Obstructive sleep apnoea (OSA) is prevalent in patients with type 2 diabetes (T2D). Since OSA and diabetes complications share common oxidative stress and inflammatory mechanisms, we hypothesized that there is a relationship between OSA and diabetic peripheral neuropathy (DPN).
Methods: T2D patients were recruited randomly from the outpatients of a tertiary diabetes centre in the UK. Patients with known respiratory disorder (including OSA) were excluded. DPN was diagnosed using the Michigan Neuropathy Screening Instrument (MNSI) (a score ≥7 on the questionnaire (MNSIq) or > 3 on the examination (MNSIe) was considered consistent with DPN). OSA was assessed using home-based portable multi-channel respiratory monitoring (Alice PDX, Philips Respironics, USA). An apnoea-hypopnea index (AHI) ≥ 5 events/hour was diagnostic of OSA (OSA+). Data are presented as median (IQR).

Table 1: Participants' characteristics in relation to OSA status. P value calculated using the Mann-Whitney U in scale variables and the Chi-square test.

	OSA+	OSA-	P value
Age (years)	61 (54-66)	54 (44-61)	0.006
Diabetes duration (years)	12.5 (6.0 -17.0)	10.5 (6.0-16.0)	0.42
BMI (kg/m^2)	33.4 (30.9-37.2)	30.3 (27.4-35.8)	0.005
Waist circumference (cm)	112.8 (106.8-123.0)	106.0 (94.8-117.4)	0.005
Neck circumference (cm)	42.3 (39.0-45.1)	38.5 (36.8-41.8)	<0.001
HbA1c (%)	7.8 (6.8-9.5)	7.8 (6.9-8.8)	0.81
Total cholesterol (mmol/l)	3.7 (3.2-4.2)	3.7 (3.4-4.5)	0.26
Triglycerides (mmol/l)	1.8 (1.2-2.5)	1.6 (1.1-2.8)	0.87
BP-systolic (mmHg)	130 (124-137)	126 (114-137)	0.04
BP-diastolic (mmHg)	78 (71-84)	79 (71-83)	0.74
Male (%)	68.5	50.7	0.04
Smoker (%)	5.5	19.7	0.012
Alcohol (%)	30.1	9.9	0.005
Insulin (%)	59.7	43.7	0.066
GLP-1 analogue (%)	14%	7%	0.28
Statins (%)	87.5	85.9	0.81
Fibrates (%)	2.7%	5.6%	0.21
ACE inhibitors (%)	47.2	43.7	0.74
Diuretics (%)	43.1	22.5	0.012
Anti-platelet (%)	79.2	63.4	0.043

Results: 143 patients were included; 50% had OSA and 46% had DPN. Participants' characteristics in relation to OSA status are summarised in Table 1. The prevalence of DPN was significantly higher in OSA+ (compared to OSA-) patients (58% vs. 32%, p=0.003). In logistic regression model (goodness to fit test p=0.002, R^2=0.34-0.46), OSA+ remained a significant predictor of DPN (OR: 3.08, 95%CI 1.01-9.38, p=0.048) after adjusting for BP, HbA1c, total cholesterol, triglycerides, diabetes duration, smoking status, alcohol intake, gender, renal function, BMI, age and the use of GLP-1 analogues, insulin, oral anti-diabetes therapy, lipid lowering therapy, anti-hypertensives and anti-platelets. Other significant predictors in the model included age (OR: 1.08, 95%CI 1.004-1.15,p=0.039), BMI (OR: 1.15, 95%CI 1.05-1.26, p=0.003), not taking a statin (OR:4.71, 95%CI 1.12-19.86, p=0.035) and total cholesterol (OR: 2.43, 95%CI 1.25-4.71, p=0.009). Log (AHI) correlated significantly with the MNSIe score (r=0.22, p=0.012). This remained the case (r=0.23, p=0.015) after adjusting for age, diabetes duration, BP, HbA1c, triglycerides, total cholesterol and renal function.
Conclusion: We describe a novel association between OSA and DPN in patients with T2D. The severity of OSA correlated significantly with the severity of DPN based on MNSIe. Planned studies aim to confirm our findings and overcome the limitations of the current study.
Supported by: NIHR

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Effects of genetic versus environmental factors on cardiovascular autonomic function and baroreflex sensitivity: a twin study
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Background and aims: Cardiovascular autonomic neuropathy (CAN) and impaired baroreflex sensitivity (BRS) can often be detected in patients with diabetes mellitus. CAN and BRS are independent predictors of cardiovascular morbidity and mortality. The heritability of cardiovascular autonomic function is not fully understood. The aim of the current study was to determine the effects of genetic and environmental factors on cardiovascular autonomic function and BRS.
Material and methods: Healthy adult twin pairs (n=101; 63 monozygotic [MZ] and 38 dizygotic [DZ] pairs; 72.5% women, 27.5% men; age: 44.3±15.5 years) were investigated. Anthropometric variables (weight, height, waist circumference) were registered, body mass index (BMI) was calculated, serum metabolic markers were measured; furthermore lifestyle and environmental background characteristics were evaluated by using questionnaires. Linear and spectral indices of heart rate variability (HRV) and BRS were determined by non-invasive methods. In resting supine position, RR interval was derived from ECG recordings, and continuous radial artery pressure was monitored simultaneously by applanation tonometry for 10 minutes. All parameters were adjusted for age and gender. Pearson correlation coefficients were calculated per zygosity (intraclass correlations) in order to determine the extent to which MZ twin pairs are more similar than DZ pairs. In addition, heritability model analyses were used for characterizing additive genetic, shared (common) and unshared (individual) environmental influences.
Results: There was no difference between BMI and waist circumference in MZ versus DZ pairs (25.9±4.9 vs. 25.8±5.9 kg/m^2, p=0.642; and 88.2±14.6 vs. 88.4±16.0 cm, p=0.986, respectively). The intraclass coefficients of correlation (r values) were low for HRV and BRS indices, in both MZ and DZ twins (LF [HRV power in low frequency range) MZ=0.140 vs. DZ=0.401; HF [HRV power in high frequency range): MZ=0.299 vs. DZ=0.225) and the heritability model analysis showed high percents of unshared environmental contribution (LF=0.816; HF=0.701). The intraclass coefficients of correlation (r values) for BRS were low in both MZ and DZ twins (BRSseq+ index: MZ=0.281 vs. DZ=0.279; BRSseq- index: MZ=0.456 vs. DZ=0.064) and the heritability model analysis showed high percents of unshared environmental contribution (BRSseq+ index=0.719; BRSseq- index=0.554). No significant changes were found after adjusting parameters for BMI values.
Conclusion: Unshared (individual) environmental but not inheritable factors have substantial influence on cardiovascular autonomic function and BRS. Accordingly, all modifiable environmental factors should appropriately be treated in order to prevent or decrease cardiovascular autonomic dysfunction and impaired BRS in patients with diabetes mellitus.
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Role of depressed cardiac autonomic activity in the impairment of heart rate recovery after exercise in diabetic patients
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Background and aims: The heart rate (HR) profile during exercise and recovery is a powerful predictor of sudden death in coronary patients and in subjects without clinically detectable cardiovascular disease. A defect of HR recovery after the termination of an exercise has been suggested to result from an impairment of the ability to increase vagal and sympathetic activity rapidly. Alterations of vago-sympathetic activity might contribute to impair HR profile during exercise and recovery in diabetic patients. The aim of this study was to examine the role of depressed cardiac autonomic activity in the impairment of HR profile during exercise and recovery in diabetic patients.
Materials and methods: We included 165 type 2 diabetic patients, 98 men and 67 women, mean age 58 yrs and diabetes duration 12.5 yrs. All of them had at least one additional risk factor (including 132 patients with hypertension) and were prospectively screened for silent myocardial ischemia (SMI), defined as an abnormal stress myocardial scintigraphy. The stress exercise test was performed on a cycle ergometer, and HR was measured at rest, at exercise peak (HRmax) and after 2 minutes of recovery. Cardiac autonomic neuropathy (CAN) was assessed using standard tests (deep-breathing, lying-to-standing and Valsalva) and was defined by at least one abnormal test.
Results: SMI, peripheral vascular disease, peripheral neuropathy and CAN were detected in 28%, 5%, 33% and 77% of the patients, respectively. HR at rest, HRmax and HR at recovery did not differ significantly in patients with or without CAN, peripheral neuropathy or vascular disease or SMI. In the total population HR decrease at recovery (% decrease from HRmax) correlated significantly with HR acceleration (% increase from rest) (r=0.532, p<0.0001). HR acceleration correlated significantly with age and HR at rest (p<0.001), and there was a trend to lower values in patients with peripheral neuropathy but HR acceleration did not differ significantly in patients with or without hypertension, peripheral vascular disease, SMI or CAN. In multivariate analysis HR acceleration was significantly associated (p=0.03) with peripheral neuropathy independently of age and HR at rest. HR decrease at recovery was slightly higher in women than in men (32±9% vs 29±10%, p=0.08), and slightly lower in patients with than in patients without CAN (30±11 vs 34±8%, p=0.143) and in those with 2 or 3 abnormal CAN function tests than in those with no or one abnormal test (29±11 vs 32±10%, p=0.148) but did not differ significantly in patients with or without hypertension, peripheral neuropathy or vascular disease or SMI. In multivariate analysis the association of HR decrease at recovery with CAN was significant (p<0.001), independently of age, gender and HR at rest. In the patients free of SMI for whom exercise was not stopped prematurely, HR decrease at recovery was significantly lower in those with CAN (p=0.02), in particular in those with 2 or 3 abnormal CAN function tests (p=0.02).
Conclusion: In high-risk but asymptomatic type 2 diabetic patients, peripheral neuropathy and CAN are major determinants of HR acceleration during exercise and HR recovery, respectively.

OP 6 Ethnic and psychosocial disparities in diabetes

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Glycaemia management of minority participants in ACCORD

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Background: Epidemiologically, African Americans (AA) and Hispanic-Americans (H) with type 2 diabetes (T2DM) have higher A1C levels than Whites (W). This difference might be due to socioeconomic factors, differences in health care access, and/or biological differences. Clinical trials may reduce the influence of some of these factors by provision of free medical care, education and medications. ACCORD trial tested the hypothesis that a therapeutic strategy targeting an A1C <6% (INT) compared to one targeting 7 to 7.9% (STD) would reduce cardiovascular events in patients with T2DM. In this post-hoc analysis of ACCORD we use A1C values to evaluate if equalization of access to health care reduces the variation in A1C across W, AA and H.
Methods: Baseline characteristics for each intervention arm were compared using ANOVA (continuous variables) and Chi-square (dichotomous variables)**.** The proportion of participants who ever reached A1C <=6.5%, for the INT and <=7.9% for STD, and among those reaching these levels, the proportion whose A1C increased to >6.5% / >7.9% at any subsequent visit, was calculated by race/ethnicity. Since most of the change in A1C in ACCORD was achieved during the first year, repeated measures models were created for A1C levels at 4-, 8-, and 12-month visits, stratified by glycaemia intervention arm. A sequential modelling approach tested the significance of effect of race/ethnicity on A1C in these models.
Results: Of 10, 251 participants, 6,393 were W, 1953 AA, and 737 H. Baseline A1C was higher for H (8.4% INT, 8.6% STD) and AA (8.5% both arms) compared with W (8.2% both arms, P <0.001 for both arms.). In the INT arm 81% of W achieved A1C <6.5% compared with 68% AA and 70% H. In the STD arm 96% W compared with 91% AA and 90% H achieved A1C <7.9%. W in INT were less likely to ↑ A1C once target was achieved (63%) than AA(74%) or H (76%); rates were similar in the STD (W 71%, AA 73%, H 67%). Mean A1C was higher at the 12 month visit in AA and H vs. W (7.8%, 7.8%, and 7.6% respectively for STD; 6.8%, 6.7%, and 6.5% respectively for INT). However, mean insulin use (units/day or units/kg/day), as well as the mean number of oral hypoglycemic agents (OHA), was similar for all groups in both arms. When baseline characteristics were accounted for in regression models, race/ethnicity remained as a significant predictor of differences in A1C for both INT (p<0.001) and STD (p=0.001).
Discussion: With similar access to diabetes care and equal use of insulin and OHA, a large percent of AA and H achieved similar A1C compared with W. However, mean A1C remained different. In regression models baseline differences in A1C, BMI, duration of diabetes, and socio-demographic differences did not alter the observation nor did on-study insulin quantity or OHA. The major strengths of the study are the large number of H and AA participants with T2DM with equal access to diabetes care as W. Weaknesses of this study include the "secondary" nature of this analysis and the lack of pre-specified culturally sensitive patient education and management. It remains to be determined whether the remaining mild differences in A1C between these ethnic groups are due to cultural or biological factors or both.
Supported by: NIH

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Screening for type 2 diabetes mellitus identifies a major burden of modifiable cardiovascular risk

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Background and aims: Population screening for Type 2 Diabetes (T2D) enables diagnosis earlier in the disease trajectory than existing approaches. If followed by sustained multifactorial intervention, vascular outcomes should theoretically improve. It is largely unknown whether population-based screening for T2D identifies cases at increased yet modifiable cardiovascular disease (CVD) risk. We aimed to compare baseline characteristics, modelled 10 year risk and estimated CVD risk reduction of two newly diagnosed T2D populations: one-screen-detected and the other clinically diagnosed.
Materials and methods: The screen detected cohort (LEADER) is an amalgamation of two population-based programmes of screening for T2D by OGTT (n=309): (i) STAR which used targeted screening of those with at least one risk factor for T2D, (ii) ADDITION-Leicester a universal screening study and RCT of CVD risk intervention in screen-detected T2D.The clinically diagnosed group, representative of T2D cases in UK clinical practice was derived from an RCT of structured education (DESMOND) (n=824). In those without prior CVD, an ethnicity adjusted Framingham equation (Ethrisk) was used to estimate 10-year CVD risk. The absolute risk reduction achievable and its plausible range were predicted using relative risk reductions for individual therapies from published trials and sensitivity analysis.
Results: Screen-detected people had statistically significant higher levels of systolic and diastolic blood pressure and total cholesterol with lower levels of BMI and HbA1c compared to those who were clinically diagnosed. Medication use was significantly higher in those who were conventially diagnosed: anti-hypertensives 60% vs. 44%, lipid lowering 41% vs. 21%, aspirin 27% vs. 16%, all p<0.0001. Mean 10 year CVD risk was significantly higher in the screen detected group (21.1% vs. 16.8%, p<0.0001). By commencing multifactorial therapy in this high risk screen-detected group the absolute risk reductions (ARR) achievable range from 4% to 10% (related to numbers needed to treat (NNT) of 10 to 25), with higher risk reductions being seen in those from a white European background compared to South Asians.
Conclusion: T2D cases identified through population screening are at high risk of CVD. At detection, this adverse CVD risk profile is often untreated, but appears potentially modifiable with existing preventative treatments. Lower estimated CVD risk reduction in south Asians suggests alternative factors may be important in determining risk within this group.

Estimation of absolute CVD risk reduction in screen detected T2D using Ethrisk

	ARR (Range)	NNT
No added effect treatment (Lipid Lowering therapy)	4.0 (2.7-5.2)	25
Additive effect: glucose, lipid and bp lowering therapies	8.5 (7.3-9.5)	12
Additive effect: glucose, lipid bp lowering therapies and aspirin	10 (9.3-11)	10
White Europeans	n=154	
No additive effect	4.6 (3.1-6.1)	21
Additive effect	9.7 (8.4-11)	***10***
Additive effect and aspirin	11.6 (11-12)	9
South Asians	n=95	
No additive effect	3.3 (2.2-4.3)	30
Additive effect	6.9 (6.0-7.7)	14
Additive effect and aspirin	8.2 (7.6-8.8)	12

Supported by: Diabetes UK & NHS

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Dispelling myths about social disparities in diabetes-related complications and mortality: Diabetes Study of Northern California (DISTANCE)

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Background and aims: In contrast to studies of health disparities in the UK and Europe which focus primarily on social class, US studies have focused much more attention on disparities associated with race/ethnicity and "vulnerable minorities". Few studies have considered how race/ethnicity and social class each relate to specific health problems. In diabetes, educational disparities in related outcomes (complications and mortality) are under-studied.

We evaluated the size and direction of educational and race/ethnic disparities in diabetes-related complications and mortality.
Material and methods: We followed a cohort of 64,419 fully-insured, patients with diabetes (19 years or older) with uniform access to integrated care (Kaiser Permanente) for 10 years (1996-2005; ~463,800 person-years). Hazard ratios (HR) were estimated from age and sex-adjusted Cox proportional hazard models (time to incident event), specified separately for all-cause mortality (MORT) and incident nonfatal and fatal complications (myocardial infarction (MI), congestive heart failure (CHF), stroke, end stage renal disease (ESRD), lower extremity amputation (AMP), after excluding those with prevalent history for each complication model.
Results: There was a significant, stepwise relationship between education and diabetes endpoints, with a 20-60% greater comorbidity and mortality (e.g., HRs for amputation: 1.6, 1.4, 1.3, 1.0 and HR for mortality: 1.3, 1.2, 1.1, 1.0 for less than high school (HS), HS graduate, some college and college grad respectively) burden for those with less than a high school education compared to college graduates. Asians had markedly lower rates of amputation (HR: 0.4 relative to Whites) than the other groups and together with Latinos, had the lowest overall morbidity and mortality rates. African Americans had the highest CHF, stroke, ESRD and amputation rates, although not significantly different than whites. Whites had the highest incidence of MI and mortality, but the lowest incidence of ESRD. Estimates were essentially unaltered after further adjustment for type of diabetes, duration of diabetes, diabetes medications, hypertension, dyslipidemia, CKD category based on GFR, albuminuria, hemoglobin A1c, prevalent MI, stroke, CHF, ESRD, and amputation.
Conclusion: Contrary to convention wisdom, there are no race/ethnic groups that are consistently at highest (or lowest) risk for diabetes complications. In contrast, low educational attainment was consistently associated with poorer health across all outcomes, independent of race/ethnicity, and may be explained by risk factors held in common for all complications (e.g., smoking, inadequate health literacy, poor medication adherence and cost barriers), thus suggesting potential candidates for interventions that could reduce disparities for combined endpoints. Future efforts to reduce social disparities among patients with diabetes should pay greater attention to socioeconomic/social class disparities, while maintaining existing attention to ethnic disparities.
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Educational disparities and the risk for mortality in patients with type 2 diabetes
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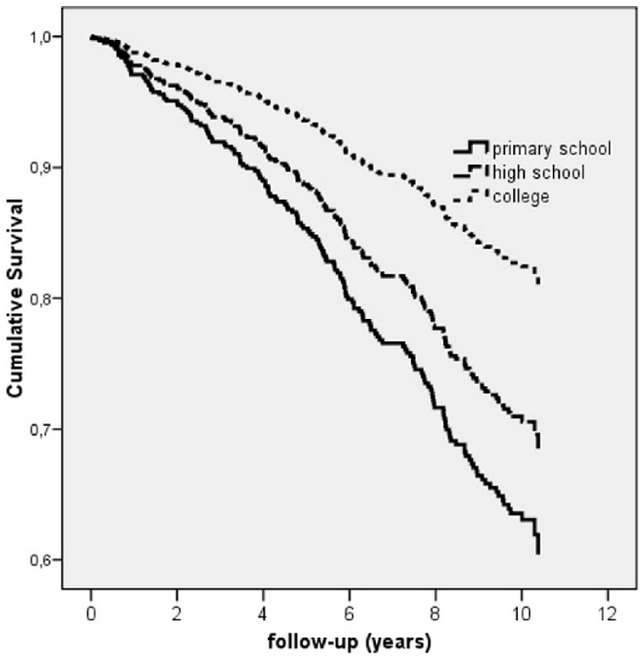
Background and aims: The relationship between socioeconomic status and mortality is clear and has also been established in studies in type 2 diabetes (T2DM). The relationship between educational level and mortality in T2DM has recently received attention in a study from the United States (US). We studied this relationship in a Dutch cohort of T2DM patients.
Materials and methods: This study is part of the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) study, in which patients with T2DM, treated in primary care, were enrolled. The ZODIAC study started in January 1998. Data on educational level were first collected on 19 May 1998, however. From this date on 858 patient were included in 1998, and educational level was known for 648 (76%) patients. The survival status (dead or alive) for each patient was recorded in January 2009. The relation between mortality and educational levels were studied with a Cox proportional hazard model. Educational level was divided in 3 categories: no education beyond primary school, high school, and college. Potential confounders for which was corrected were: age, sex, body mass index, smoking status (yes/no), macrovascular complications (yes/no), diabetes duration and working status.
Results: After a median follow-up time of 9.7 years, 367 out of 858 patients had died, the cause of death was known for 350 patients: 40% died from cardiovascular disease and 23% died from malignancies. Compared to patients who finished primary school, patients who had gone to college, had a Hazard Ratio (HR) of 0.42 (95% CI 0.19-0.91) for total mortality, and patients who had gone to secondary school had a HR of 0.75 (95% CI 0.51-1.11) for total mortality (see figure 1). No significant relationship between educational level and cardiovascular and cancer mortality was found.
Conclusion: Educational level was associated with total mortality even after adjustment for working status. Patients with no education beyond primary school had an increased risk for total mortality compared to those who had gone to college. The risk of mortality differs substantially according to educational level among persons with T2DM in the Netherlands and confirms the results recently found in the US.



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Socio-economic impact on the quality of diabetes mellitus type 2 care: can we improve the outcomes?
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Background and aims: According to the European Commission, the executive body of the European Union, "social and economic deprivation involves persons, families or populations whose economic, cultural and social conditions are limited to a level below the minimal life standard accepted in their country of domicile." This definition embraces large populations with diabetes mellitus type 2. In order to counteract the impact of deprivation and poverty on treatment of diabetes mellitus in those populations, the specific patterns and mechanisms of poor socio-economic status existing in the real world of diabetes mellitus care should be objectively delineated. Therefore, the study presented below was conducted.
Materials and methods: Using standardized socio-medical and economic questionnaires, the study was conducted on a cohort of 1,050 persons with diabetes mellitus type 2 hospitalized in 2005-2008 in the public diabetological centre (Department of Diabetology, Bródnowski Hospital, Warsaw). Their socio-economical and health status characteristics were diagnosed in a standardized way using appropriate questionnaires and a grading system containing four categories: 1, economic; 2, psychological and social; 3, educational, and 4, medical.
Results: In the cohort of 1,050 hospitalized diabetic type 2 persons under study, 282, or 29%, were found to have social, economical or psychological and educational deprivation and poverty not meeting local minimal life standards as defined by the Central State Statistical Office. The specific components of the deprivation and poverty standards that were analyzed were: 1, character of family and social support; 2, profession and employment; 3, level of formal education; 4, monthly income, help of social organizations; 5, fear and depression; 6, practice of self-control, and 7, frequency of planned medical outpatient visits. Using these parameters, the existence or non-existence of deprivation and poverty was diagnosed and produced two groups of patients: 1, those afflicted by deprivation and poverty (282 out of 1,050 cases, or 27.9%), and 2. those not afflicted (768 out of 1,050 cases, or 72.1 %). The medical diagnoses in both subgbroups are presented below.
Conclusion: The social, economical, psychological and educational deprivation and poverty acts as a separate risk factor for the level of diabetes

mellitus type 2 control. It is reflected by the differences in morbidity, due to the chronic complications of diabetes mellitus, and also by the existence of additional chronic diseases associated with diabetes mellitus. The definition and diagnosis of the deprivation and poverty in diabetes type 2 based on four components of this life status: social, economic, psychological and educational is very practical for counteraction plans. Each component may have individual expression and may require a separate counteractive plan, both clinical and administrative.

Medical parameters, percentage in both groups	Deprivation/ Poverty present	Deprivation/ Poverty not present	p<0.05
Level of HbA1c			
X (SD)	10.6 (2.8)	8.2 (1.6)	*
Lack of self-control practice	86	40	*
Retinopathy - all stages	52	40	*
Nephropathy - all stages	26	5	*
Ischemic diseases			
Heart	72	64	-
Brain	62	46	*
Lower extremities	54	38	*
Neuropathy	60	36	*
Existence of additional chronic disease	89	72	*
Emotional disorders, depression	40	39	-

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Ethnic differences in the prevalence and recognition of depression in a primary care population with and without type 2 diabetes

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Background and aims: Results from meta-analyses suggest that the prevalence of depression is higher in people with type 2 diabetes compared to those without. However a number of methodological limitations in the literature remain including the use of inadequate control groups and a failure to control for potentially confounding factors. The present study aimed to determine the prevalence of screen detected and prevalent (currently managed) depression in a multiethnic primary care population with and without type 2 diabetes in the UK. In addition the study aimed to examine ethnic differences in the prevalence of depression between South Asian (SA) and White European (WE) people with type 2 diabetes and to assess the recognition of depression in primary care.

Materials and methods: Consecutive primary care attendees were screened for depression using the depression subscale of the Hospital Anxiety and Depression Scale (HADS-D) during routine appointments in primary care. Demographic and medical data were also extracted from participants' primary care records.

Results: Complete data were available for 860 adults with type 2 diabetes (560 SA and 300 WE) and 643 without diabetes. No significant differences in the prevalence of depressive symptoms were observed between people with and without type 2 diabetes (HADS-D≥ 8= 28% vs.29%, P>0.05; HADS-D≥11= 17% vs. 17%, P>0.05). Higher rates of probable and/ or major depression were observed in SA people with type 2 diabetes compared to WEs: 32% vs. 22%, P= 0.006 (HADS-D≥8) and 13% vs. 10%, P= 0.166 (HADS-D ≥11). Using a cut-off of ≥8 on the HADS-D, the ethnic difference also persisted when controlling for age, gender, deprivation and the presence of one or more co-morbidity or diabetes-related complication. In those scoring ≥11 on the HADS-D, rates of unrecognised depression were higher in those with diabetes compared to those without (83% vs. 70%, P=0.004), and were significantly higher in SA in comparison to WE both in those with type 2 diabetes (90% vs. 63% P=0.018) and those without (77% vs. 50%, P=0.002).

Conclusion: In contrast to previous research, the findings showed no significant difference in risk for depression in those with diabetes compared to those without, suggesting that the association between depression and diabetes may be less robust than previously acknowledged. However in people with type 2 diabetes, higher rates of depression were observed in SAs in comparison to WEs. Furthermore our results also indicate that depression is seriously under-diagnosed in people with type 2 diabetes, most acutely in SAs, suggesting a need to improve methods of identifying depression in these patients.

OP 7 The effects of insulin beyond glycaemia

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Insulin in patients with acute myocardial infarction and diabetes - friend or foe? A report from the DIGAMI 2 study

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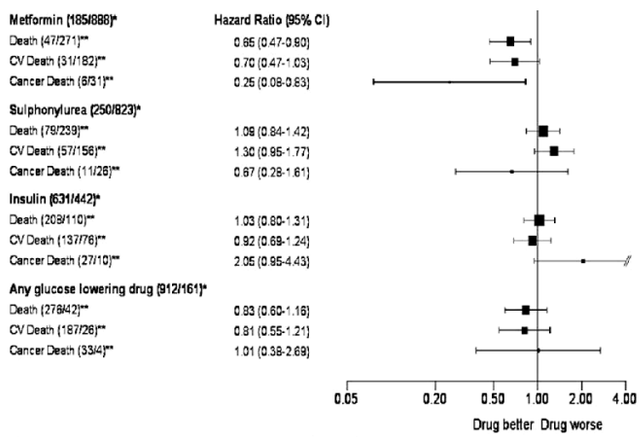
Background and aims: The aim of the present report, a substudy to DIGAMI 2, is to analyze long-term mortality and morbidity in relation to different glucose lowering agents in patients with myocardial infarction and type 2 diabetes.
Materials and methods: In DIGAMI 2 patients with type 2 diabetes and suspect myocardial infarction were randomised to Group1) insulin based treatment; Group 2) insulin in-hospital followed by conventional glucose control; Group 3) conventional treatment. They were treated according to the protocol for 2.1 years and in an extended part of the trial followed up to 8.3 years (median =4.1). Long-term follow-up data was available in 1145 patients, 91.3% of the original DIGAMI 2 cohort.
Results: The total mortality was 34%, (72 % cardiovascular). Cox regression analysis did not show any difference in total or cardiovascular mortality between treatment groups. The total number of fatal malignancies was 37 with the highest risk in group 1. Hazard Ratio compared with group 2:1.83 (95%CI 0.90-3.71; p=0.096) and to group 3: 3.57 (95%CI 1.22-10.39; p=0.02). Patients on insulin treatment had a higher risk of non-fatal events (OR 1.90 (95%CI 1.38- 2.6; p=<0.0001) but not increased mortality (OR 1.30, 95%CI 0.94-1.80; p=0.11). Metformin was associated with a lower mortality (HR 0.65, 95%CI 0.47-0.90) and a lower risk of death of malignancies (HR 0.25, 95%CI 0.08-0.83; figure).
Conclusion: Patients with diabetes and myocardial infarction have a very poor prognosis. The drug used for glucose control appears to have a prognostic impact. Insulin was associated with an increased risk of non-fatal cardiac events and death due to malignancies while metformin seemed to be protective.



Supported by: the Swedish Heart-Lung Foundation, AFA Insurance

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Safety analysis of basal insulins: mitogenic potency and receptor binding

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Background and aims: The receptor binding profile of exogenous insulin may provide important information regarding mitogenicity; for example, higher affinity to the insulin-like growth factor-1 receptor (IGF-1R) has been linked to increased cellular proliferation. Furthermore, sustained signalling from the receptor might also increase cellular proliferation. Preclinical analysis has demonstrated a comparable mitogenic profile between insulin detemir (IDet) and neutral protamine Hagedorn insulin. This analysis of insulin receptor (IR) and IGF-1R binding was undertaken to evaluate the mitogenic characteristics of basal insulin analogues.
Materials and methods: Three insulin analogues were compared with human insulin: IDet, insulin glargine (IGlar) and an experimental insulin, X10. Two experiments were performed: first mitogenic potency relative to human insulin was established through proliferation of two different cell lines - human mammary epithelial cells (HMEC), and L6 myoblasts modified to overexpress human insulin receptors (L6-hIR). Then, receptor binding for IDet and IGlar was measured using both cell membranes and solubilised receptors, with binding curves generated to calculate the ratio of IGF-1R binding: IR binding profile relative to human insulin. Both isoforms of insulin receptor (A and B) were used.
Results: The mitogenic potency of IDet relative to human insulin was comparatively low for both cell lines (see Table). Data for IGlar were varied, with the HMEC cell line showing a high potency, and the L6-hIR line a relatively low potency. For X10, a consistently high potency in both cell lines was observed. When the ratio of IGF-1R:IR binding was calculated for IDet, both IR isoforms, and both techniques showed a similar ratio relative to human insulin: 0.7 (IR-A) and 1.3 (IR-B) for membrane experiments and 0.4 (both IR-A and B) for solubilised receptors. For IGlar, ratios were increased relative to human insulin: 16.2 (IR-A) and 20.5 (IR- B) for membrane preparations, and 10.7 (both IR-A and B) for solubilised receptors.
Conclusion: Compared with human insulin, IDet has a lower mitogenic potency, although further parameters of molecular safety (for example, sustained signalling from the IR or IGF-1R) may also be of note when evaluating the mitogenic potential. Our data show a similar balance between IGF-1R and IR binding for IDet and human insulin without evidence of significant differences in proliferation, supported by previous studies.

Table. Mitogenic potencies of study insulins relative to human insulin

Cell line	Functional receptor	Human insulin	Insulin detemir	Insulin glargine	X10
HMEC	IGF-1 and insulin	100%	16.9%	650%	812%
L6-hIR	Insulin	100%	9.2%	49%	744%

Supported by: Novo Nordisk

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Changes in the concentration of serum C-peptide in type 2 diabetes during long-term continuous subcutaneous insulin infusion therapy

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Background and aims: Type 2 diabetes is characterized with impaired beta cell function and reduced beta cell mass which deteriorate over time. To see if beta cell function can be improved in type 2 diabetic patients through long-term continuous insulin infusion (CSII) therapy, we examined changes in serum C-peptide levels during the treatment.
Materials and methods: We discontinued oral antidiabetic drugs (OADs) and applied CSII therapy to subjects with type 2 diabetes who had failed to control hyperglycaemia with OADs and/or insulin injections (number, 34 with 59% of male; age, 56.8 ± 10.8 years; duration, 9.3 ± 7.3 years; HbA1c 7.4 ± 1.8%). Blood samplings were performed yearly for 4 years at 12 hour-overnight fasting and 30 and 120 minutes after ingestion of a standard mixed meal (500 kcal; carbohydrate 52.9%, lipid 30.4%, protein 16.7%) with at least 9 hours cessation of CSII.
Results: During the 4 year-CSII treatment, the mean HbA_{1c} significantly decreased from 7.4 ± 1.8% to 5.6 ± 0.4% ($p < 0.01$, Fig.1) and the mean serum C-peptide level at 120 minutes after meal ingestion significantly increased from 5.97 ± 2.71 to 7.46 ± 2.68 ng/ml ($p < 0.05$, Fig.1). The C-peptide increment from fasting to 120 minutes after meal also increased by 1.06 ± 1.97 ng/ml after 1 year-CSII therapy, 0.79 ± 1.91 ng/ml after 2 years, 1.61 ± 2.62 ng/ml after 3 years, and 4.27 ± 2.17 ng/ml after 4 years, respectively.
Conclusion: The resolution of glucotoxicity and maintenance of euglycaemia through long-term CSII therapy may contribute to the restoration of β-cell function in terms of serum C-peptide level after meal in type 2 diabetic patients.

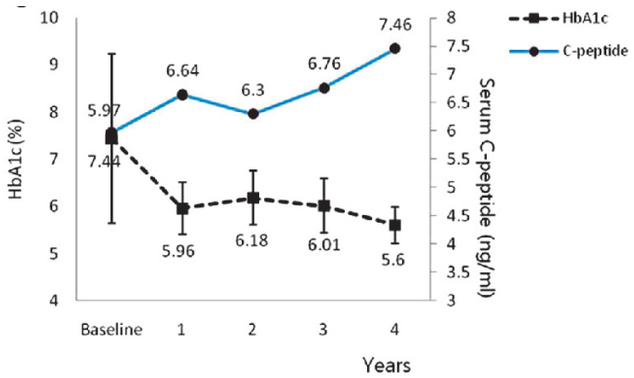

Fig.1. Changes in the mean HbA1c and the mean serum C-peptide concentration at 120 minutes after ingestion of a standard mixed meal (500 kcal) during 4 year-continuous subcutaneous insulin infusion therapy in patients with type 2 diabetes.
Supported by: Novo Nordisk

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Body weight increase during the first year of insulin treatment in patients with type 2 diabetes mellitus; a systematic review and meta-analysis of different insulin regimens

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Background and aims: Insulin treatment increases body weight in type 2 diabetes mellitus. Aim of this study was to define body weight increases during the first year of insulin treatment as a function of treatment modalities.
Materials and methods: We considered 51 randomized, parallel group studies with 15202 patients, lasting 12 - 52 weeks, published as full papers during years 1991 - 2009. Of each study we analyzed number of patients, duration of treatment, co-treatment with oral agents, insulin regimen (basal, bis in die, prandial), glycemic target, daily insulin doses, HbA1c and its changes, proportion of patients with hypoglycemia, body weight and its changes. Pooled-random effects of estimates of insulin regimen on body weight increase, compared with other regimens, were calculated using the Der Simonian and Laird models.
Results: Body weight increased 2.1±0.16 kg (range -0.85 - +7.5 kg, 95% C.I. 1.812-2.468), with no influence of basal body weight, lower with basal than with bis in die regimen (OR 0.42, 95% C.I. 0.25-0.59), and with no difference basal vs prandial, nor bis in die vs prandial regimen. Body weight increase was directly related (weighted regressions) to duration of treatment (r = .514,p = 0.0001), insulin dose (r = .313, p = 0.0012), HbA1c (r = .331, p = 0.0005), HbA1c change (r = .402, p = 0.0001), glycemic target (r = .203, p = 0.0462), hypoglycemia (r = .453, p = 0.0001), and nocturnal hypoglycemia (r = .281, p = 0.0434). At stepwise regression analysis (model 1: r = .783, F = 36.865; model 2: r = .735, F = 41.597), independent variables duration of treatment, HbA1c (or HbA1c change), and frequency of hypoglycemia, were significantly correlated to body weight increase (dependent variable).
Conclusion: These data indicate a slight and progressive increase of body weight in patients with type 2 diabetes during the first year of insulin treatment, related to insulin regimen, basal HbA1c, its changes, and to frequency of hypoglycemia.

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Effects of insulin detemir and NPH insulin on renal handling of sodium, fluid retention and weight in type 2 diabetic patients

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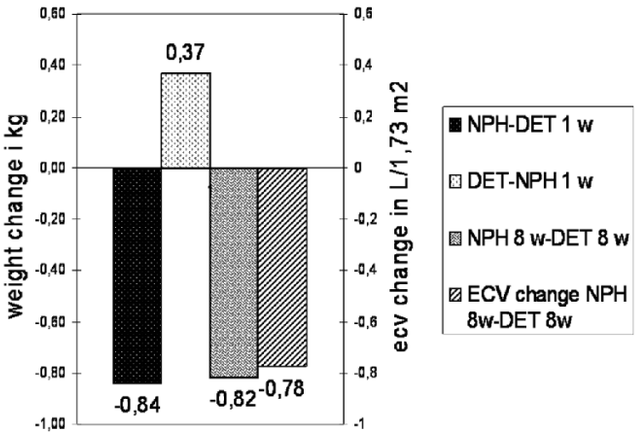
Background and aim: In insulin treated type 2 diabetic patients, insulin Detemir (DET) seems to induce less weight gain compared to NPH insulin (NPH). The mechanism behind this weight-reducing effect of DET is unknown, but it has been proposed that the cause is reduced fat accumulation or reduced sodium/water retention. Due to the albumin binding and reduced glomerular filtration, and thereby reduced tubular action of DET, we tested the hypothesis that type 2 diabetic patients should increase their urinary sodium excretion (UNaE) and thereby reduce extracellular volume (ECV) and body weight (weight) when insulin treatment was changed from NPH to DET.
Material and methods: In a randomised, open-labelled, two-way cross-over study, 24 patients with type 2 diabetes and normal urinary albumin excretion (mean age: 62 yr, mean diabetes duration: 15 yr, gender 17/7 (M/F), mean HbA1c: 7.6%) were included. They were first treated with either NPH or DET for 8 weeks. In the second period, they were changed to the other insulin for 8 weeks. In a third 1-week period, they were changed back to the first insulin. At the end of each 8-week period ECV, weight and body composition (DEXA scan) was measured. In the last week of each 8 week period and on day 1, 3, and 5 in the first week after therapy change UNaE was determined. Weight was measured after 1 week. Twenty-four hour blood pressure was measured 6 times in the course of the study.
Results: At the end of 8 weeks weight was reduced by 0.8(±0.2 kg)(mean±SEM) after DET treatment compared with NPH (paired t-test: p<0.01) A non significant reduction in ECV of 0.8(±0.5 L/1.73m²)(paired t-test: p= 0.14) was found. The weight loss occurred already after 1 week 0.8(±0.2kg)(paired t-test: p<0.001) and simultaneously a transient increase in UNaE was observed (Kruskal-Wallis test: p=0.07). HbA1c increased by 0.6%(±0.1) after 8 weeks of treatment with DET (paired t-test: p<0.001) in spite of a slightly increased insulin dose, but average blood glucose measured during the first week was similar during the two treatments. In addition, 24 h blood pressure and body composition remained similar during the two treatment periods.
Conclusion: DET induces a significant and sustained weight loss, first observed within the first week after changing therapy from NPH. It was simultaneous with a trend towards a transient increase in UNaE and a reduction in ECV. The weight loss thus seems to be related to changes in fluid volume and may reflect changed insulin action in the kidneys.



Supported by: Novo Nordisk

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Basal insulin therapy improves indexes of endothelial damage and regeneration in type 2 diabetes. A randomised cross-over trial comparing detemir vs glargine

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Background and aims: Endothelial damage is the leading mechanism of diabetic macroangiopathy. In diabetes, endothelial regeneration is also impaired owing to reduced levels of endothelial progenitor cells (EPCs). We tested the hypothesis that therapy with basal insulin analogues influences endothelial damage and regeneration in type 2 diabetes. Since the two basal analogues have different pharmacological properties, detemir and glargine were directly compared.
Materials and methods: This was a 3-month randomized cross-over trial comparing insulin detemir once daily versus glargine once daily added to oral agents in poorly controlled (HbA1c>7.0%) type 2 diabetic patients with cardiovascular disease (ClinicalTrial.gov NCT00699686). Insulin titration was aimed at achieving fasting plasma glucose between 5.0 mmol/l (90

mg/dL) and 6.1 mmol/l (110 mg/dL) as soon as possible after enrollment. At baseline, at cross-over (3 months) and at study end (6 months), we measured HbA1c, EPC levels (as an indicator of endothelial regenerative capacity) and serum concentrations of VCAM-1, ICAM-1 and E-selectin (as indicators of endothelial damage). EPCs were defined as CD34+KDR+ or CD133+KDR+ or CD34+CD133+KDR+ cells and measured by flow cytometry. We also recorded body weight and hypoglycemic episodes.
Results: Forty-two patients completed the study: 21 followed the glargine-detemir (GD) schedule and 21 the detemir-glargine (DG) schedule. Patients were on average 66.1±1.2 year old and 74% were males. Baseline data did not differ between the two groups in terms of demographic and anthropometric parameters, HbA1c, complications and concomitant treatment. At cross-over, patients in the GD schedule had a lower HbA1c as compared to patients in the DG schedule (p=0.040). Even if this difference in HbA1c was lost at study end, the overall HbA1c effect was in favor of glargine (p=0.016). Incidence of hypoglycemia (mild and severe together) and weight gain were lower during detemir than during glargine therapy. ICAM-1, VCAM-1 and E-selectine were all significantly reduced at crossover as compared to baseline and further decreased at study end, irrespectively of the treatment schedule. At cross-over, the levels of all EPC phenotypes did not change as compared to baseline, while CD133+KDR+ and CD34+CD133+KDR+ cells significantly increased (by 68% and 139%, respectively) at study end in both groups.
Conclusion: Basal insulin therapy improves indexes of endothelial damage and regeneration in type 2 diabetic patients with cardiovascular disease. Glargine and detemir do not differ in their endothelial effects, but detemir achieved a similar endothelial protection with lower weight gain and hypoglycemic episodes, albeit at the expenses of a slightly higher HbA1c. These results might have implications for therapy of aging type 2 diabetic patients with cardiovascular disease.
Supported by: LIBRA project, Novo Nordisk

OP 8 Continuous glucose monitoring - a promise of improvement?

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Glucose control in adults during a 1-year randomised controlled trial comparing sensor-augmented pump therapy and multiple daily injection therapy: STAR 3 study

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Background and aims: Recent studies have demonstrated overall improvement in glucose control with use of CGM. Sensor augmented pump (SAP) therapy combines an insulin pump with real-time integrated continuous glucose monitoring. We hypothesized SAP would improve glucose control (A1C and AUC >180 mg/dL) without increasing hypoglycemia.
Materials and methods: STAR 3 is a 1-year multicenter randomized controlled trial which compared SAP therapy using insulin aspart to multiple daily injection (MDI) therapy using insulin glargine and insulin aspart in 329 adult (age 19-70 years) and 156 pediatric (age 7-18 years) subjects with type 1 diabetes. All subjects wore a "blinded" sensor for 6 days at baseline, and the MDI subjects wore subsequent blinded sensors for 6 days at 6 months and one year. Preliminary results for the adult cohort (age 19-70 years) are given for: A1C, mean sensor glucose, area under the curve (AUC), and severe hypoglycemia.
Results: The primary endpoint of change in A1C from baseline to 1 year showed the decline in mean A1C levels was significantly greater in the SAP group compared to MDI group (SAP: from 8.3±0.5% to 7.3±0.7%; MDI: from 8.3±0.5% to 7.9±0.9%; treatment difference -0.6%; 95% CI, -0.77 to -0.45; p<0.001). The Table demonstrates that mean sensor glucose was statistically similar at baseline but significantly lower in the SAP group compared to the MDI group at 6 months (p<0.01) and 1 year (p<0.01). The improvement in glycemia with SAP was not associated with an increase in severe hypoglycemia (p=0.58) or AUC <70 mg/dL at 1 year (p=0.82). There were no between group differences in incidence of DKA (p=0.38) or weight gain (p=0.20). A greater proportion of adult subjects in the SAP group (57/166, 34.3%) reached ADA targets for A1C compared to the MDI group (19/163, 11.7%; p<0.001). AUC for sensor glucose values >180 mg/dL at 6 months and 1 year were significantly reduced in the SAP group compared to the MDI group (p<0.001).
Conclusion: SAP achieved a greater reduction in A1C and AUC>180 compared to the MDI group without an increase in hypoglycemia over a period of a year.

Table. Mean Sensor Glucose, Hyperglycemia, and Hypoglycemia, SAP and MDI Treatment Groups

	Baseline		6 Months		1 Year	
	SAP	MDI	SAP	MDI	SAP	MDI
Mean sensor glucose (SD), mg/dL	179.9 (28.9)	178.7 (27.6)	157.5* (25.4)	171.1 (31.5)	158.6* (25.7)	174.2 (31.7)
AUC>180 (SD)	28.9 (17.8)	28.0 (17.3)	16.0* (12.6)	24.7 (18.5)	16.1* (12.8)	26.0 (19.5)
ΔAUC>180	NA	NA	-12.9	-3.3	-12.8	-2.0
AUC<70 (SD)	0.3 (0.5)	0.3 (0.5)	0.3 (0.5)	0.4 (0.6)	0.3 (0.4)	0.3 (0.6)
ΔAUC<70	NA	NA	0	0.1	0	0

*, p<0.01 compared to MDI group; SAP, sensor-augmented pump; MDI, multiple daily injection; AUC, area under the glucose concentration-time curve; Δ, change from baseline; NA, not applicable

Supported by: Medtronic, Inc.

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Effects of continuous glucose monitoring on glycaemic control in subjects with type 1 diabetes delivering insulin via pump or multiple daily injections: a prospective study

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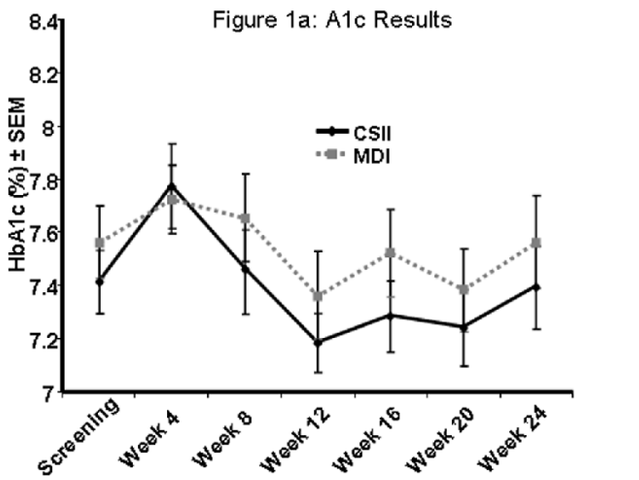
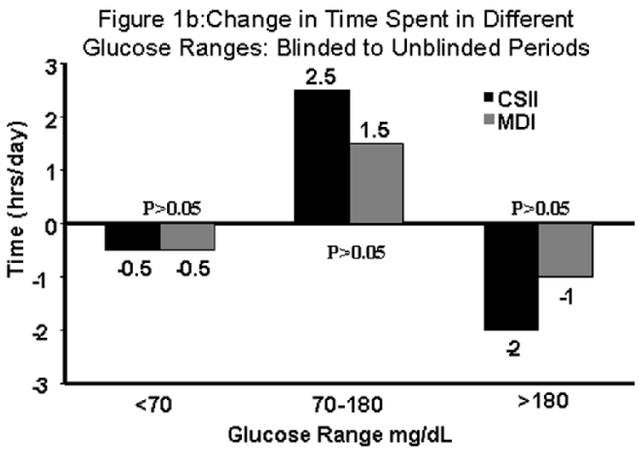
Background and aims: There are no long-term prospective data describing the differential effects (if any) of continuous glucose monitoring (CGM) when used by patients with type 1 diabetes (T1D) who deliver insulin via multiple daily injections (MDI) as compared to those on continuous subcutaneous insulin infusion (CSII) pump therapy. This study assesses the long term effectiveness of CGM (DexCom SEVEN*PLUS) in subjects with T1D and compares the changes in glycemic control by CSII vs. MDI cohorts.
Materials and methods: Sixty adults with T1D were enrolled in this 6-month study; 30 subjects were on CSII, and 30 delivered insulin via MDI. For the first 4 weeks, all subjects were blinded from CGM values/trends/alarms; thereafter all subjects were unblinded for the remaining 20 weeks (real-time CGM data provided). A1C was measured during screening and at 4-week intervals throughout the study. Per the results of the JDRF CGM trials and the pre-specified analysis, population for this study included subjects who utilized CGM at least 6 days per week (86% of the time).
Results: Mean (±SD) age was 36 (±11) and 39 (±8) years; duration of diabetes was 22 (±11) and 23 (±10) years; and screening A1C was 7.61% (±0.76) and 7.63% (±0.68) for CSII and MDI groups, respectively (p>0.05). Seventeen patients (57%) in each group utilized CGM for at least 6 days each week. Both groups similarly improved A1C values (Figure 1a) and time spent in target range glycemia (Figure 1b). Both groups reduced time spent in hypoglycemia (180 mg/dL) was reduced by one and two hours per day in the MDI and CSII groups, respectively.
Conclusion: Similar improvements in glucose control can be achieved with real-time CGM in compliant T1D patients using either CSII or MDI.





Supported by: A research grant through the University of Colorado Denver from Dexcom, Inc

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Closed-loop insulin delivery using subcutaneous infusion and glucose sensing, and equipped with a dedicated safety supervision algorithm, improves safety of glucose control in type 1 diabetes

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Background and aims: The reduction of blood glucose excursions outside a near-normal range is a main goal for the improvement of control of type 1 diabetes. The modulation of insulin delivery according to blood glucose levels and variations can be achieved by using closed-loop (CL) insulin delivery systems based on continuous interstitial glucose monitoring (CGM), subcutaneous insulin infusion (CSII), and control algorithms. We investigated the new concept of modular model-based safety supervision aiming the prevention of hypoglycaemia while keeping blood glucose level in a safe range.
Materials and methods: Six type 1 diabetic patients (5 M/1F), duration of diabetes 31+/- 7 years, age 43 +/- 7, A1c 7.5 +/- 0.9 %, and BMI 25 +/- 3, treated by CSII for 7.6 +/- 3.9 years, were enrolled in a trial performed at the CHU Montpellier Clinical Research Center using Navigator CGM and OmniPod insulin pump infusing lispro to compare fully-automated CL control equipped with a dedicated safety supervision algorithm to physician-supervised CSII. The trial was composed of two periods during which each insulin delivery mode was tested in a randomised order from 2 pm to 8 am the next day. The patients performed a 30-min exercise bout on a cyclo-ergometer at 50% VO2 max from 4 pm, took a 70g CHO-meal dinner at 7 pm as well as a 20g CHO-snack at 10:30 pm, and slept from 11 pm to 8 am. Reference blood glucose was measured using YSI every 30 min from 2 to 11 pm and every hour from 12 to 8 am. Primary endpoint was the number of hypoglycaemic events according to symptoms and YSI glucose value below 70 mg/dl. Secondary study endpoints included % time spent outside a safe glucose range of 70-180 mg/dl, mean blood glucose level and maximal blood glucose difference during the entire trial periods and during exercise and following 2.5 hours, 2 hour-post dinner period, and night-time from 9pm to 8 am.
Results: Compared to CSII, CL control reduced the incidence of hypoglycaemia two-fold: from 13 to 6 events. While mean blood glucose levels (mg/dl) were similar for both periods: 155 +/- 14 (CSII) vs. 145 +/- 14 (CL), percent time spent < 70 mg/dl and > 180 mg/dl were both lower during CL vs. CSII: 2.4 +/- 0.9 vs. 4.7 +/-1.2 and 18.0 +/- 6.5 vs. 30.1 +/- 5.7, respectively. Maximal blood glucose difference (mg/dl) was significantly tighter at exercise and recovery time during CL: 84 +/- 16 vs. 123 +/- 21 (p=0.046).
Conclusion: Our preliminary data based on the first 6 patients enrolled in this pilot study show that a modular model-based safety supervision system results in fewer hypoglycaemic events and reduces the duration of blood glucose excursions outside the target range 70-180 mg/dl, as well as the amplitude of glucose variations associated with exercise in type 1 diabetes. Upcoming larger clinical trials will confirm the benefits of added safety provided by this algorithm.
Supported by: JDRF

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Overnight closed-loop glucose control following consumption of alcohol in adults with type 1 diabetes

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Background and aims: Modern management of insulin in type 1 diabetes (T1D) uses flexible insulin therapy to minimise restrictions on daily life. By reducing the capacity for hepatic gluconeogenesis, alcohol consumption increases the risk of delayed hypoglycaemia. Even for those patients who reduce overnight insulin after drinking, hypo- or hyperglycaemia can follow, especially where alcohol is consumed with larger meals in social settings. We have tested a closed-loop (CL) or artificial pancreas system linking off-the-shelf

continuous glucose monitoring (CGM) and continuous subcutaneous insulin infusion (CSII) using a purpose-built model predictive control (MPC) algorithm. We recently demonstrated that overnight CL in adults significantly increased time spent in euglycaemia and reduced time in hypoglycaemia. We have now examined the efficacy and safety of CL compared with standard CSII on overnight glycaemic control, after evening alcohol intake accompanying a large dinner.

Materials and methods: Twelve adults with T1D treated by CSII (F 7, age 37.2±9.9yrs, BMI 26.8±4.2 kg/m^2, T1D duration 19.7±9.7yrs, duration on pump 1.9±2.5yrs, HbA1c 7.8±0.7%; mean±SD) were studied on 2 separate nights in random order, receiving either overnight CL (used from 2200 until 1200 the next day) or usual CSII therapy (using a similar Deltec Cozmo insulin pump on both nights). On CL nights, subcutaneous CGM values from the Freestyle Navigator were fed into an MPC algorithm every 15min, which calculated the infusion rate of Aspart (adjusted manually). On CSII nights, subjects used the Cozmo pump to deliver their usual insulin rates. On both occasions, between 2030 and 2200, a mixed meal (100g-carbohydrate) was ingested accompanied by 0.75g/kg ethanol as 13% white wine (mean volume consumed 564±133ml), with a pre-prandial insulin bolus using the subject's usual bolus calculator. Plasma glucose was measured every 15min to assess CL performance.

Results: As shown in the table, CL significantly increased the time spent in target glucose range and reduced glucose variability (plasma glucose SD). CL reduced the high blood glucose index, a composite measure of duration and severity of hyperglycaemia. One episode of severe hypoglycaemia occurred with CSII and none during CL. Notably, improved glycaemic control with CL was achieved without a difference in the average insulin dose infused.

Conclusion: CL can provide significantly better overnight glucose control than conventional CSII after alcohol and large meal consumption. CL has the potential to improve efficacy and safety of flexible insulin regimens.

Comparison of overnight plasma glucose control during CL and CSII

	CL	CSII	P
Time in target 3.9-8.0mmol/l (% of total time)	**72±15**	50±23	**0.002**
Time > 8.0mmol/l (% of total time)	23±16	37±26	**0.042**
Time < 3.9mmol/l (% of total time)	5±7	13±13	NS
Mean overnight glucose (mmol/l)	6.8±0.8	7.0±1.5	NS
SD overnight glucose (mmol/l)	1.8±0.5	2.3±0.7	**0.031**
High blood glucose index	1.8±1.6	3.2±2.6	**0.016**
Low blood glucose index	1.4±1.2	2.9±2.6	NS
Mean overnight insulin infusion (U/h)	0.9±0.4	0.9±0.4	NS

Supported by: Diabetes UK, NIHR, JDRF

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Comparison of total annual direct costs among Swedish residents with poorly controlled type 1 diabetes: standard care versus real-time continuous glucose monitoring

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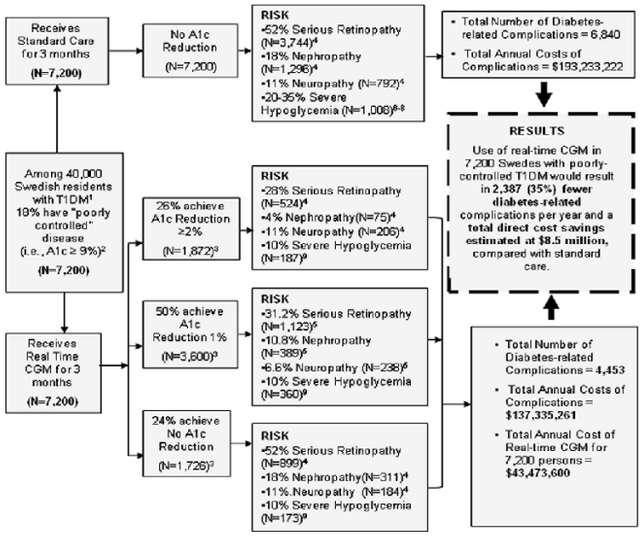
Background and aims: Among the 40,000 Swedish residents diagnosed with type 1 diabetes (T1DM), 18% (7,200) have poorly-controlled disease (defined as HbA_{1c} ≥9%) and are at high risk for developing diabetes-related complications. Randomized clinical trials (RCTs) have shown that real-time continuous glucose monitoring (RT-CGM) significantly improves HbA_{1c} (A1c) levels among those with T1DM. We developed a decision-tree model to compare anticipated rates and costs of diabetes-related complications among a hypothetical group of 7,200 Swedish subjects with poorly-controlled T1DM who received RT-CGM or standard therapy (ST).

Materials and methods: T1DM prevalence was obtained from the National Board of Health and Welfare and A1c levels from the Swedish Diabetes Registry; A1c improvement among those with poorly-controlled T1DM who used RT-CGM was obtained from a RCT; rates of microvascular complications in Swedish subjects with T1DM were derived from the Stockholm Diabetes Intervention Study and the Diabetes Complications and Control Trial. Rates of severe hypoglycemia in T1DM were based on observational studies of Swedes and a RCT of RT-CGM. Annual direct medical costs of were obtained from published Swiss (serious retinopathy) and Swedish (nephropathy, peripheral neuropathy, severe hypoglycemia) economic analyses and reported as 2009 USD. Costs for 7-day, continuous, RT-CGM were provided by a manufacturer of RT-CGM devices.

Results: Among the 7,200 who received RT-CGM, 26% achieved a 2-point A1c reduction, 50% a 1-point reduction, and 24% no improvement at 1 year; among a comparable group of 7,200 Swedish subjects who received ST, A1c did not change from baseline. Among those who received RT-CGM versus ST, 3,949 and 5,832, respectively, experienced microvascular complications, and 720 and 1,440-2,520, respectively, severe hypoglycemia. The total annual reduced direct costs associated with diabetes-related complications for those who received RT-CGM versus ST ranged from $43.3 to $45.9 million. The cost of receiving RT-CGM was $43.5 million ($6,038 per person). Thus, RT-CGM reduced 2,603-3,683 diabetes-related complications over 1 year ranging from a net break-even to a cost savings of $2.4 million.

Conclusion: In this decision-tree model, use of RT-CGM by 7,200 Swedes resulted in 2,603-3,683 (36-44%) fewer diabetes-related complications per year compared with ST at an estimated reduction in the direct cost of diabetes-related complications ranging from $43.3 to $45.9 million. The model demonstrates that this technology is a cost-effective means of reducing diabetes-related complications among Swedish persons with poorly-controlled T1DM. Because not all direct costs for diabetes-related complications (e.g., macrovascular events) were included in the model, RT-CGM may be associated with even greater cost savings relative to ST.



Supported by: DexCom, Inc.

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Accuracy of a continuous glucose monitoring system (CGMS): still room for improvement

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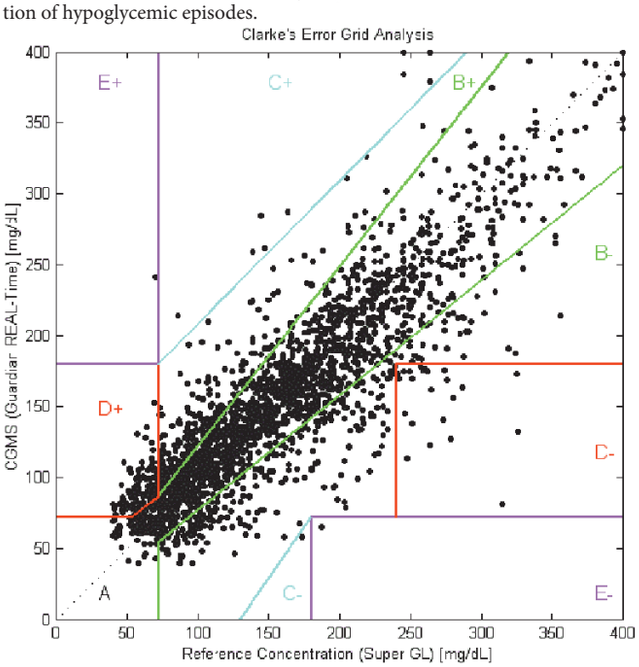
Background and aims: Clinical trials have shown improvements in glycemic control through CGMS in people with type 1 diabetes (T1D), but systematic investigations of the accuracy of CGMS are scarce. We studied the performance of the Guardian Real Time CGMS in 17 T1D (6 females, mean age 43 (26-61) years, HbA1c 8.3 (7.6-8.9)%, all on basal-bolus insulin therapy).

Materials and methods: During two in-house stays of 7 days each with standard meals (identical for both periods) patients were connected to CGMS (hypoalarm setting 70 mg/dl with a predictive alarm 10 min earlier). Reference measurements using a laboratory device (Super GL glucose analyzer, Hitado, Möhnesee, Germany) were done at least every 4 hours and in case of hypoglycemic symptoms or a CGMS hypoalarm. All reference measurements were used for CGMS calibration.

Results: In total, 2328 paired data points were obtained covering a glucose range of 40 - 400 mg/dl. Mean absolute deviation was 24.5±24.0 mg/dl, mean relative absolute deviation was 18.3±18.7%. Frequent calibration did not seem to improve accuracy, relative absolute deviations for values obtained within 1h after calibration was 18.8±18,6%. A Clarke Error Grid analysis showed 93% of paired values to be in zones A (67.4%) and B (25.6%), but also 6.4% in zone D (figure). Reference measurements indicated 145 episodes of hypogly-

cemia (blood glucose (BG) < 70 mg/dl) of which only 43 were detected with CGMS. Sixty additional CGMS hypoglycemic alarms were not confirmed, i.e. reference measurements showed BG values > 90 mg/dl. Thus, while CGMS had high specificity (97%) and good negative predictive value (95%) for hypoglycemia, positive predictive value (42%) and sensitivity (30%) were poor.
Conclusion: In conclusion, further improvements in the accuracy of CGMS measurements seem to be necessary in particular for a more reliable detection of hypoglycemic episodes.



OP 9 GWAS and their follow-up: analytical, technological and experimental developments

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Genome-wide association analysis in over 187,000 individuals identifies 14 loci contributing to variation in fat-distribution
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Background and aims: Obesity is an increasing public health issue, but not all forms of obesity carry the same risk. Individuals with high waist-to-hip ratio (WHR) have an increased risk of Type 2 Diabetes (T2D), hypertension, heart disease, stroke and certain cancers. WHR is one of the primary measures of fat distribution and has a substantial heritability (~50%), independent of overall adiposity. Hitherto, the genetic variants that contribute to variation in WHR have not been well characterized.
Materials and methods: To detect common variants, we performed a meta-analysis of 32 genome-wide association studies comprising >77,000 individuals of European ancestry as part of the GIANT consortium. We tested ~2.8 million imputed and genotyped SNPs for association with WHR using an additive model, adjusted for age and BMI and sex.
Results: Our discovery analysis identified 16 independent loci associated with WHR with >110,000 individuals). In our analysis combining discovery and follow-up studies 14 loci reached genome-wide significance (p<5x10-8). We confirmed the known locus near LYPLAL1 (1q41; p=4.9x10-19) and identified 13 novel associations, including four loci that suggest an overlap with developmental processes and T2D risk: RSPO3 (6q22; p=1.8x10-40), near VEGFA (6p12; p=5.9x10-25), TBX15 (1p11; p=8.7x10-25), and near ADAMTS9 (3p14; p=9.8x10-14). RSPO3 may regulate embryonic angiogenesis via the Wnt signaling pathway. TBX15 is differentially expressed between subcutaneous and visceral fat and the expression is correlated with WHR. VEGFA is a growth factor that has been suggested to play a role in diabetic nephropathy and retinopathy. ADAMTS9 is significantly associated with T2D, possibly mediating an effect through decreased insulin sensitivity of peripheral tissues. We find a directionally consistent enrichment of associations (above what would be expected by chance) for each single trait (P nominal <0.05) with increased triglycerides, LDL-cholesterol, fasting insulin, and HOMA-IR. We also observed a marked gender difference in our results; 7 of the 14 loci showed a stronger association in women than in men.
Conclusion: Taken together, these results promise to enhance our knowledge of underlying biological pathways involved in fat-distribution and propose a genetic overlap with metabolic risk and T2D. Hopefully these advancements will support functional and translational advances in the management of obesity through development of novel diagnostic and therapeutic options.
Supported by: The Wellcome Trust

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Genetic susceptibility for obesity increases the risk of type 2 diabetes and is modified by macronutrient intakes
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Background and aims: Genome-wide association studies (GWAS) have identified at least 16 novel single nucleotide polymorphisms (SNPs) associated with BMI or obesity. The aims of this study were to examine whether these SNPs associate with obesity, type 2 diabetes or cardiovascular disease (CVD) in Swedish population and to investigate if dietary intake (relative intakes of fat, carbohydrate, protein and fiber) or physical activity level modify the genetic effect, measured as a genetic risk score (GRS), on BMI and body composition.
Materials and methods: In total 17037 individuals from the Malmö Preventive Project (MPP) were genotyped and a weighted GRS was created. Association between SNPs and GRS and BMI, obesity, type 2 diabetes and CVD were studied cross-sectionally and prospectively after a mean of 23 years follow-up. A total of 9023 non-diabetic subjects participated in the population-based Malmö Diet and Cancer Study (MDCS) with dietary data from a modified diet history method and with leisure time physical activity determined and

were included to GRS x diet interaction analyses. Bioelectric impedance analysis was used to estimate body composition.
Results: 14 SNPs associated with BMI or related traits and were included in GRS. A 13% (10-17%) increased risk of obesity for each increased quintile of GRS was observed ($p=3x10^{-16}$). Individuals in the highest vs. lowest GRS had a 29% (14-46%) increased risk of type 2 diabetes ($P= 5x10^{-5}$ and P=0.006 after adjustment for BMI). GRS was not significantly associated with CVD (p=0.12). The effect sizes of GRS quintiles on BMI were 2.2, 1.9 and 1.7 times higher comparing low carbohydrate-, low fiber and high fat intake tertiles to high carbohydrate, high fiber and low fat intake tertiles (p=0.016, p=0.022 and p=0.084 for interaction, respectively). Interactions were strongest on lean mass (p=0.002, p=0.001 and p=0.049). Effect sizes of GRS on BMI, fat mass or lean mass did not significantly differ between tertiles of leisure time physical activity (p=0.44, p=0.88 and p=0.26, respectively).
Conclusion: GRS of 14 BMI/obesity SNPs associates with obesity and type 2 diabetes. Diets low in carbohydrates and fiber and/or high in fat may accentuate genetic susceptibility for obesity, particularly accumulation of fat-free mass in males. The fairly modest combined effect size on BMI by the so far identified BMI/obesity SNPs can partially be explained by the effect sizes being modified by dietary intakes.
Supported by: Swedish Medical Research Council, Påhlsson Foundation, an EFSD Clinical Research Grant, LUDC

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A genome-wide association analysis of over 75,000 individuals identifies gender-specific effects for fasting glycaemic traits
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Background and aims: Meta-analyses of genome-wide association (GWA) studies for glycaemic traits up to date have described 16 loci influencing fasting glucose (FG) and two loci - fasting insulin (FI) levels. FG levels are tightly regulated, but may differ between genders. Gender differences in prevalence of IFG (impaired fasting glucose) and IGT (impaired glucose tolerance) are well known: IFG is more common in men and IGT in women but the mechanisms for these differences are unknown. Within the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) we aimed to investigate gender-specific differences in genetic regulation of FG/FI levels, since such effort in a large enough sample was lacking.
Materials and methods: To identify gender-specific genetic effects we performed gender-stratified meta-analyses of 36 genome-wide association (GWA) studies informative for FG and FI within MAGIC in up to 32,993 and 27,870 non-diabetic men and 42,149 and 34,940 non-diabetic women, respectively. Age information was provided for ~69,600 individuals from 32 cohorts. Mean age in males was 53.5(SD=10.2) and in females was 51.1(SD=10.8) years.
Results: Among previously described loci *DGKB-TMEM195I* demonstrated the largest differential effect estimates (heterogeneity Q P=0.026, I^2=80%) stronger in men (β=0.031 [SE=0.004], $P=9.1x10^{-14}$) than in women (β=0.019 [SE=0.004], $P=1.1x10^{-7}$). Gender-specific heterogeneity for *MTNR1B* was also high (Q $P<0.05$, I^2=75%) with higher effect estimates for men (β=0.080 [SE=0.005], $P=3.7x10^{-56}$) than for women (β=0.067 [SE=0.004], $P=3.3x10^{-51}$). Effects estimates in women were higher for *C2CD4B* and *GCKR*, where the gender heterogeneity was close to high, but not significant (I^2~60%, Q $P>0.05$). *G6PC2* and *ADCY5* showed moderate and *GCK, PROX1, SLC30A8* low between-gender heterogeneity for FG ($I^2 \leq 50\%$ and $I^2<25\%$, respectively). We couldn't detect gender-specific differences in the FG effect estimates for *MADD, FADS1, CRY2, ADRA2A, GLIS3*, *SLC2A2 TCF7L2* and at *GCKR* for FI (heterogeneity I^2=0%). In FG meta-analysis we uncovered a novel locus showing strong heterogeneity (Q P=0.055, I^2=73%) near *PCSK1* with women showing β=0.023 [SE=0.003], ($P=3.0x10^{-9}$) and men β=0.012 [SE=0.004], (P=0.008); overall β=0.018 [SE=0.003], ($P=9.0x10^{-10}$). Meta-analysis of FI has revealed higher effect estimates in men (β=0.034 [SE=0.006], $P=3.3x10^{-8}$) compared to women (β=0.018 [SE=0.006], P=0.001) at rs860598 (overall β=0.024 [SE=0.004], $P=3.6x10^{-9}$, heterogeneity Q P=0.042, I^2=76%) in *IGF1* locus, showing a stronger association than the previously described variant rs35767 (overall $P=4.7x10^{-8}$, linkage disequilibrium r^2=0.77).
Conclusion: We showed that some genetic loci have greater contribution to the fasting glycaemic traits variability in men (*DGKB, MTNR1B, IGF1*) than in women and vice versa (*PCSK1*) owing to differential gender-specific physiological mechanisms of regulation of glycaemic traits. We also unveiled a novel FG locus near *PCSK1*. This analysis showed the importance of deep investigation of between-gender differences for quantitative phenotypes related to type 2 diabetes. Well powered GWA analyses should investigate the gender contribution to the quantitative traits variability in a systematic manner.
Supported by: European Commission under the Marie Curie Intra-European Fellowship

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Identification of additional type 2 diabetes susceptibility loci through large-scale replication using "Metabochip": early result
I. Prokopenko[1,2], A. Mahajan[3], V. Lagou[1,2], N. Robertson[1,2], W. Rayner[1,2], A. Kumar[1], C.J. Groves[2], D. Buck[1], A. Doney[4], C.N.A. Palmer[5], A.D. Morris[5], A.P. Morris[1], C.M. Lindgren[1,2], M.I. McCarthy[1,2], for the DIAGRAM consortium;
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Background and aims: Over 30 common variant signals influencing type 2 diabetes (T2D)-susceptibility have been identified so far. However, replication efforts to date have focused on limited numbers of the most strongly-associated signals and have failed to exploit fully the information provided by the well-powered genome wide association (GWA) meta-analyses available for stage 1 discovery. The Metabochip - a custom iSELECT array containing ~195,000 SNPs - has been designed to support large-scale follow-up of putative associations for T2D and other metabolic and cardiovascular traits, and to enable the fine-mapping of known loci.
Materials and methods: This analysis is based on Metabochip data from 3,185 T2D cases and 3,569 controls (from the UK T2D Genetics Consortium sample recruited in Tayside, Scotland) that were successfully called (Gencall v1.1) and passed quality control (QC). Of 185,802 Metabochip SNPs passing QC, 4,821 were included in this analysis because they capture the top ~5,000 independent autosomal signals from the DIAGRAM (v3) GWA meta-analysis (12,057 T2D cases, 56,071 controls, all of European-descent). Association analysis was performed under an additive model with adjustment for 3 principal components to account for sample substructure.
Results: We observed directional consistency for all published T2D-loci (and for 11 novel autosomal loci derived from the most recent DIAGRAM+ consortium effort) including variants at *TCF7L2* ($P<10^{-14}$); *SLC30A8*, *KCNQ1*, *FTO* ($P<10^{-4}$); *KCNJ11* and *IRS1* ($P<5x10^{-3}$). We compared overall patterns of replication between the DIAGRAM stage 1 discovery data and the UK-T2DGC follow-up results for 4,333 independent SNPs represented in both samples. Of these, 2,468 SNPs showed directionally consistent effects (binomial $p<10^{-19}$) with 217 of the 2,468 also showing nominal replication (i.e. $p<0.05$, same effect direction). Only 79 of 4,333 SNPs had $p<0.05$ in the opposite direction (binomial $p<10^{-18}$). Despite the modest size of this first follow-up sample (compared to the discovery set), joint analysis of DIAGRAM+ Stage 1 and UKT2DGC Stage 2 data revealed several signals near to or exceeding genome-significance, including a locus near *ARL15* (ADP-ribosylation factor-like 15) on chromosome 5p15 (UKT2DGC $P=10^{-4}$, meta-analysis $P=3.2\times10^{-8}$, OR=1.11[95%CI 1.07-1.16]).
Conclusion: These preliminary data obtained from the Metabochip are consistent with a long tail of common variant association signals of modest effect contributing to T2D-susceptibility. Metabochip-genotyping is currently underway in over 30,000 T2D cases and 50,000 controls of European descent. Follow-up on this scale should add considerably to the tally of proven T2D-susceptibility loci. Even in this modestly scaled initial follow-up effort, we have identified one novel T2D-susceptibility signal mapping to chromosome 5p15.

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Fine-scale mapping of type 2 diabetes susceptibility loci by imputation and conditional analysis

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Background and aims: Up to 30 T2D susceptibility genes have been identified in the past few years from genome-wide association scans (GWAS). In this study, we focus on three regions implicated by GWAS in European populations - CDKAL1, CDKN2A/B, and FTO. To facilitate identification of the causative variants within these genes, we have undertaken fine-scale mapping at greater marker density, and explore the possibility of multiple causative signals in each gene.
Materials and methods: We used 1924 T2D cases and 2938 controls previously studied in a 500K GWAS by the Wellcome Trust Case Control Consortium (WTCCC). We used imputation (with IMPUTEv2) to estimate the genotypes of an additional 3157 SNPs (frequency>1%) across these regions. This method uses a single, hierarchical, approach to combine a primary reference set of 112 phased CEU chromosomes from the 1000 Genomes project (August 2009 release) with a secondary panel of an additional 298 phased CEU and Tuscan chromosomes from the HapMap Phase 3 project (release 2). We analysed each SNP under an additive genetic model using SNPTEST v1.1.4. We performed conditional analyses, adjusting for the most significant SNP at each stage using a forward selection strategy.
Results: At CDKAL1 we identified a cluster of imputed SNPs (the best being rs10440833, risk-allele frequency (RAF) =0.32, allelic OR=1.28, $P=7.3x10^{-8}$), with stronger T2D association than the previously reported lead SNPs (rs9465871 and rs10946398) from the WTCCC GWAS. This cluster is focussed on a 27kb haplotype block flanked by rs9465871 and rs10946398. We found a second independent signal at imputed SNP rs6930283 (P=0.0005, RAF =0.32) 100kb 5' to the primary signal. In CDKN2A/B, the most significant signal from our imputed data was seen at rs7018475 (RAF=0.30, allelic OR=1.37, $P=1.2x10^{-9}$), at the 3' end of the 9kb haplotype block containing rs10811661, the previously reported lead SNP. Rs10811661 remains associated (with P=0.004) after accounting for rs7018475, suggesting it represents a largely independent effect. At FTO, our imputed genotypes provide further evidence for association with BMI (in T2D cases, age & sex-adjusted) across the 50Kb haplotype block implicated in the WTCCC GWAS. The best signal in our study comes from imputed SNP rs11646715 ($P=3.5x10^{-6}$). Conditioning the analysis on rs11646715 completely abolishes the evidence for association (P>0.2 at all SNPs) across the region. The strongest association with T2D from our imputed data in FTO comes from rs1421086 (allelic OR=1.27, $P=1.8x10^{-8}$), comparable with previously identified lead SNPs rs7193144, rs8050136 and rs9939609. Conditioning association analysis on imputed SNP rs1421086, or these three lead SNPs, largely ablates the evidence for T2D association across the 50Kb haplotype, with the best residual signal being imputed SNP rs1861867 (P=0.001-0.005) in each case.
Conclusion: Imputation using data from the 1000 Genomes and HapMap3 projects is a valuable tool for fine-mapping loci, identified from GWAS, with near complete coverage of common variants. Using this approach, we have more precisely localised the causative variants in CDKAL1, CDKN2A/B and FTO. These newly identified imputed variants are candidates for further genotyping and functional assessment.
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Insights into glucokinase regulatory protein regulation from the cellular and kinetic characterisation of rare coding variants

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Background and aims: Genes containing common variants of significant phenotypic effect may also harbour rare variants (Minor Allele Frequency [MAF] <5%) of large effect. The common P446L glucokinase regulatory protein (*GCKR*; protein name GKRP) variant is associated with decreased risk of type 2 diabetes, lower fasting glucose and insulin, and increased triglyceride levels. GKRP binds and inhibits glucokinase (GCK) in the liver, sequestering GCK in the nucleus in the fasting state. Increased glucose levels trigger GCK dissociation from GKRP, nuclear GCK export, and GCK activation. Functional analysis suggests the P446L variant acts through decreasing F6P-mediated inhibition of GCK, increasing liver glucose uptake and disposal. The aim of this study was to identify and functionally characterise rare *GCKR* variants for cellular localization and GCK inhibition to provide insight into mutation severity and direct both clinical and genetic follow-up.
Materials and methods: Exonic *GCKR* sequencing was performed in 664 individuals from the multi-ethnic NIH ClinSeq project using standard methodology**.** Nonsynonymous variants (published and novel) were introduced into a YFP-tagged GKRP expression plasmid. Wild-type (WT) and variant YFP-GKRP were transiently transfected into HeLa cells and cellular localisation assessed microscopically 24 hours post-transfection. CFP-tagged GCK was co-transfected with YFP-GKRP to assess GCK interaction and nuclear sequestration. Recombinant human GCK and both WT and Q234P GKRP were generated. Q234P-GKRP and WT-GKRP inhibition of GCK activity and regulation by F1P and F6P were determined spectrophotometrically using an NADP+ coupled assay.
Results: Twelve (nine novel) nonsynonymous variants were identified. Seven variants were observed in only one individual. Excluding P446L, variants had MAFs ranging from 0.2-1% in the ClinSeq cohort. Of novel variants, only one (Q234P) was identified in the 1000 Genomes project. WT YFP-GKRP localized primarily to the nucleus and was necessary and sufficient to sequester CFP-GCK to the nucleus. Of 18 variants tested, 12 (including P446L) had increased cytoplasmic: nuclear fluorescence compared to WT. Of the 12 variants with increased cytoplasmic fluorescence, six (including Q234P) did not sequester CFP-GCK to the nucleus. Kinetic analysis showed no difference in inhibition of GCK activity by WT- or Q234P-GKRP over a glucose concentration range of 0-100mM. However, response to 20-500μM F1P (n=38; $5x10^{-9}<p<0.02$) and 10-500μM F6P (n=41; $1x10^{-6}<p<0.02$) were both significantly diminished with Q234P-GKRP.
Conclusion: A large proportion (67%) of nonsynonymous *GCKR* variants disrupt nuclear localization and/or interaction with GCK in a cellular context. Since there is no apparent variant clustering it suggests that many changes in GKRP primary structure can affect global folding and three-dimensional structure. Prioritisation of variants for additional genetic analysis and targeted phenotyping may be aided not only by determining the MAF in appropriate ethnic groups but also by selecting variants such as Q234P that show both altered GCK sequestration and kinetic parameters.
Supported by: NIH GPP; MRC

OP 10 Lipids in and out of context

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Exercise-induced reduction in liver fat is accompanied by improvements in vascular function in non-alcoholic fatty liver disease

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Background and aims: Non-Alcoholic Fatty Liver Disease (NAFLD), the hepatic manifestation of the metabolic syndrome is characterised by the accumulation of triglycerides in the liver and is associated with liver-related morbidity and mortality as well as increased cardiovascular risk. Exercise training is recommended as a therapeutic technique to reduce hepatic fat in NAFLD patients, yet the efficacy of exercise training remains equivocal. Flow mediated dilation (FMD), the increase in conduit artery diameter in response to increases in flow, provides information regarding endothelial cell health and is an early barometer of cardiovascular disease. Endothelial function has been shown to improve with exercise training in young healthy, older sedentary and obese individuals but has not been investigated in the NAFLD population. Therefore, the aim of this study was to examine the effect of regular exercise on intrahepatocellular lipid (IHCL) content and endothelial function in NAFLD patients.
Materials and methods: 6 sedentary NAFLD patients aged 56±9 yrs underwent a 16-week supervised exercise training program (30-45mins, 3-5 times per week). Whole body magnetic resonance imaging with proton magnetic resonance spectroscopy was used to determine IHCL levels prior to and following training (n=5). Fasting glucose, lipids, AST and ALT, brachial artery FMD, responses to glyceryl trinitrate (GTN) and cardiorespiratory fitness (VO_{2peak}) were also assessed before and after training. Differences between baseline and post-training data were analysed using paired t-tests. Data are described as mean±SD.
Results: IHCL significantly reduced by 28.7% following exercise training compared to baseline (21.4±13.7 vs 17.2±13.8%; P=0.01), this was accompanied by a significant improvement in FMD (7.3±3.8% vs 3.8±1.6%, P=0.02). Liver enzymes also significantly improved following exercise training; ALT was reduced to 28±6U/l from 39±9U/l at baseline (P=0.008). Fitness improved by 20.2% (23.50±4.56 vs 28.52±8.82ml/min/kg; P=0.06) and body mass (79.6±10.9 vs 77.2±12.0kg; P=0.04) and AST (P=0.01) were significantly reduced following exercise training. No significant changes in GTN or lipid profiles were evident after exercise training.
Conclusion: This is the first study to demonstrate that both IHCL and endothelial function significantly improve following exercise training in NAFLD patients. This indicates that regular exercise has concomitant therapeutic effects on both excess hepatic fat and cardiovascular disease in these high risk patients. The exercise-mediated improvement in IHCL and FMD was accompanied by clinically significant reductions in liver enzymes and body mass. These data strongly support the efficacy of exercise training as a non-pharmacological management strategy in NAFLD and suggest as well as improving liver function, exercise may decrease the risk of heart disease and stroke in these patients.
Supported by: an EFSD Clinical Research Grant

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Study of GPIHBP1 expression in human adipose tissue, correlation with lipid parameters and regulation by rosiglitazone

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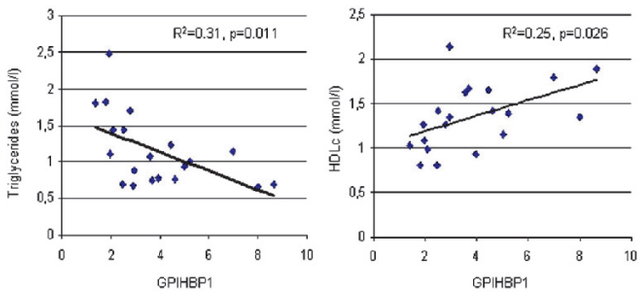
Background and aims: Glycosyl phosphatidyl inositol HDL binding 1 (GPIHBP1) gene encodes for a new endothelial surface binding site for lipoprotein lipase (LPL), the key enzyme for intravascular lipolysis of triglyceride-rich lipoproteins. Mice lacking GPIHBP1 manifest severe hyperchylomicronemia with lipolysis defect. Similarly to LPL, GPIHBP1 is expressed in adipose tissue, skeletal muscle and heart. GPIHBP1 expression is stimulated by rosiglitazone (PPARγ agonist) in mice. GPIHBP1 involvement in triglyceride (TG) metabolism is not clearly established in Humans. The aim of this study is to investigate GPIHBP1 expression levels in human adipose tissue of healthy and type 2 diabetic subjects, its correlation with lipid parameters, and its potential regulation by rosiglitazone.
Materials and methods: We studied GPIHBP1 expression by quantitative RT-PCR in sub-cutaneous adipose tissue (SCAT) and/or visceral adipose tissue (VAT) biopsies of 20 healthy subjects and of 17 type 2 diabetic subjects after 12 weeks of placebo or rosiglitazone (4 mg/day).
Results: In healthy subjects, GPIHBP1 is expressed in both SCAT and VAT, with a significant correlation between the 2 tissues (R^2=0.49, p=0.001). We find an inverse significant correlation between TG and GPIHBP1 expression in SCAT (R^2=0.31, p=0.011) (Figure) and in VAT (R^2=0.26, p=0.021). Conversely, HDLc is positively correlated with GPIHBP1 expression only in SCAT (R^2=0.25, p=0.026) (Figure) (VAT: R^2=0.11, p=0.144). Other lipid parameters, age and body mass index are not significantly associated with GPIHBP1 expression neither in SCAT or VAT. Additionally, GPIHBP1 expression is correlated with TG in type 2 diabetic subjects in SCAT (R^2 =0.41, p=0.034). Moreover, its expression is significantly increased by rosiglitazone compared to placebo (+ 32.6 %, p=0.022).
Conclusion: We show for the first time in Humans that GPIHBP1 is expressed in both SCAT and VAT and that its expression is inversely correlated with TG in healthy and type 2 diabetic subjects and regulated by a PPARγ agonist. These results suggest an important role for GPIHBP1 in TG metabolism in Humans.



Correlation between lipid parameters (TG, HDLc) and GPIHBP1 expression in SCAT

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Cardiac lipid content increases upon exercise-induced elevation of plasma fatty acid concentrations

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Background and aims: Increasing evidence suggests that excessive lipid accumulation in cardiac tissue hampers cardiac function, predisposing to cardiomyopathy and heart failure. Cardiac energy status (PCr/ATP) has strong prognostic value in heart failure patients and might be an early marker of disturbed cardiac metabolism, and it has been suggested that cardiac lipid accumulation reduces the energy status. We have previously shown that elevated levels of circulating FA, induced by exercise in the fasted state, lead to increased levels of lipids in non-active skeletal muscle. However, it is unknown if the heart responds similarly. Here, we aimed to investigate if elevating plasma FA by exercise results in an increased IntraCardiomyoCellular Lipid (ICCL) content, and if this influences cardiac function and energy status.
Materials and methods: Nine male subjects (age: 25 ± 1.2 y, BMI: 23.0 ± 1.0 kg/m2) underwent proton cardiac Magnetic Resonance Spectroscopy (1H-MRS) (Intera, 1.5T, Philips Healthcare) in the morning in the fasted state to determine ICCL. Subsequently, subjects cycled for two hours at 50% of maximal performance. ICCL was measured directly after exercise and again four hours post-recovery, together with systolic function (by multi-slice cine-MRI) and cardiac energy status (by 31P-MRS). All subjects underwent this procedure twice, once while fasted, and once while ingesting glucose during both exercise and recovery (bolus: 1.4g/kg, 8 x 0.35 g/kg), which has previously been shown to prevent elevation of plasma FA levels. For cardiac 1H-MRS, a 6 cm3 VOI was placed in the septum of the heart (PRESS, TR = 4s, TE

= 26ms, n = 128). Signal acquisition was ECG-triggered to end-systole and respiratory-gated using a pencil beam navigator. Lipid content is expressed as percentage of the CH2 peak, relative to the unsuppressed water signal. For cardiac 31P-MRS, ISIS was used for localization to the left ventricle (TR = 3.6 s, n = 192).
Results: Plasma FA concentrations were increased three-fold during exercise and nine-fold during recovery in the fasted condition compared with the glucose condition ($p<0.01$). Fat oxidation was significantly higher in the fasted condition compared with the glucose-supplemented condition. ICCL was elevated at the end of the fasted test day (from 0.26 ± 0.04 to 0.44 ± 0.04 %, $p<0.01$), while it did not change when glucose supplementation was given (from 0.32 ± 0.03 % to 0.26 ± 0.05%, $p=0.3$). There were no changes in ICCL directly after acute exercise. Furthermore, PCr/ATP was decreased by 32% in the high plasma FA condition compared with the low FA condition ($n=6$, $P=0.014$). However, in the high FA condition, the ejection fraction post-recovery was higher compared with the low FA condition (63 ± 2 % vs 59 ± 2 %, $p=0.018$).
Conclusion: The increase in ICCL in the fasted condition suggests that plasma FA concentrations play an important role in determining ICCL. Furthermore, increased ICCL was associated with a decreased cardiac energy status (lower PCr/ATP), which is in line with the "lipotoxic" action of cardiac lipids. However, ICCL seems not to be the main determinant of cardiac function.

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Ectopic lipid accumulation in the heart of severe obese patients: relationship to cardiac function and visceral fat
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Background and aims: Epicardial fat is an ectopic fat depot which development is associated with coronaropathy. Its closed anatomic situation to the adjacent myocardium allows local paracrine interaction, suggesting that EF could have a functional role in obesity related cardiomyopathy. Myocardial lipid deposition is also a marker of ectopic fat accumulation, that could lead to impaired myocardial function, as suggested by preliminary results limited to midly obese patients. In this study, we quantified with a 70 cm bore 3 teslas MRI, EF volume (EFV) and myocardial triglyceride content (MTGC) in morbid obese patients, in order to determine whether these ectopic fat depots accumulate similarly, are linked to metabolic disorders or to myocardial function.
Materials and methods: 57 subjects with normal LV function and no coronary artery disease, (33 lean, and 24 obese patients (9 with type 2 diabetes) (BMI= 21.4 ± 2 and 42.3 ± 6 kg/m^2)) were studied by MRI, and underwent anthropometric, biological and visceral abdominal fat (VAT) assessment. EFV was assessed volumetrically, and MTGC using H^1 spectroscopy.
Results and conclusions: EFV and MTGC were positively correlated ($r=0.47$, $p<0.0001$) even after VAT was accounted for ($p=0.005$), and were both strongly correlated with age, BMI, waist circumference (WC), and VAT. EFV was the only ectopic depot negatively correlated with thigh circumference/BMI ratio ($r=-0.68$, $p=0.0002$), suggesting that the development of EF is related to the lack of increase of subcutaneous fat with weight gain. We observed that the two cardiac depots did not accumulate similarly, as the ratio EFV/MTGC increased with diabetes and decreased with physical activity. Moreover, EFV and MTGC statistically increased between lean and obese patients ($p=0.002$, $p=0.006$), and there was a more twofold increase in EFV between obese and diabetics (123 ± 11 vs 240 ± 46 mL, $p=0.003$). EFV and MTGC were both strongly associated with metabolic syndrome ($p<0.0001$), and with metabolic risk markers (triglycerides $r=0.59$, $p<0.0001$; $r= 0.35$, $p=0.01$, HOMA-IR $r=0.44$, $p<0.003$; $r= 0.38$, $p=0.02$, and CRP $r=0.39$, $p<0.03$ and $r= 0.49$, $p=0.005$, respectively). However, when including VAT and EFV or MTGC together, only VAT remained statistically associated with metabolic parameters ($p<0.01$). Furthermore, EFV and MTGC were associated with parameters of cardiac function. Interestingly, MTGC was the only independent parameter associated with normalized end-diastolic and systolic volumes ($r=-0.37$, $p=0.02$ and $r= -0.42$, $p=0.01$), cardiac index ($r=-0.34$, $p=0.04$), and normalized stroke volume ($r=-0.34$, $p= 0.03$). To conclude, the developement of EFV and MTGC is not similar. EFV and MTGC are linked to metabolic disorders, but VAT remains the best predictor of metabolic risk. Lastly, our work reinforces the importance of cardiac steatosis, in that MTGC is associated with early myocardial dysfunction. It suggests that in an earlier stage of non ischemic cardiomyoapthy lipid oversupply to cardiomyocytes may lead to lipotoxic injury on myocardium.

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Role of pancreatic fat in beta cell dysfunction and diabetes
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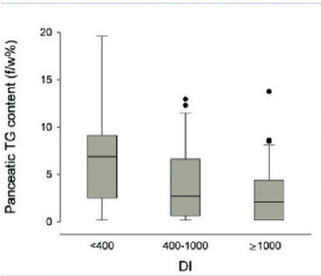
Background and aims: Fat accumulation in the beta-cells is thought to contribute to the pathogenesis of beta-cell dysfunction and the development of diabetes, at least in animal models. We explored the relevance of this hypothesis in the pathogenesis of diabetes in humans by evaluating the relationship between total pancreatic fat content (panTG) and insulin secretion in vivo in human volunteers.
Materials and methods: We enrolled 91 volunteers, with and without diabetes, with an average age of 41.2 years, and body mass index (BMI) of 32.9 kg/m2. PanTG and visceral fat area were measured in vivo by magnetic resonance spectroscopy and imaging, respectively; beta-cell function was measured using a frequently sampled glucose tolerance test. Acute insulin response (AIR) is a measure of first phase insulin secretion, while the disposition index (DI) is a measure of insulin secretion adjusted for the prevailing insulin sensitivity. DI is a good indicator of disease progression, as it decreases from normal (>1000) in patients with normal glycemia, to very low (<400) in those who have progressed to diabetes. Non-normally distributed variables were transformed. To compare panTG across DI groups while adjusting for age and BMI we used ANCOVA analysis. Multivariate regression analysis was performed for the dependent variable AIR, most representative models are shown in the figure.
Results: PanTG increased significantly across groups as DI worsened, even after adjusting for age and BMI ($p=0.01$). PanTG was highest in the group with the lowest DI, at 7.37 (+/-5.9) f/w% (panel A). PanTG, along with age, hip/waist ratio, and BMI were significant independent predictors of AIR (panel B - model 2). Visceral fat area was not a significant predictor of AIR when analyzed along with the same variables (panel B - model 3).
Conclusion: PanTG content is a significant contributor to beta-cell dysfunction, and ultimately development of diabetes, independent of visceral fat.



A: Pancreatic TG content by DI groups.

Model	Variable	Parameter Estimate	P-value	Adjusted R^2
Model 1	Age	-0.404	0.0002	0.304
	Hip/waist	-21.898	0.037	
	BMI	0.604	0.0003	
Model 2	Age	-0.355	0.0008	0.364
	Hip/waist	-24.708	0.0151	
	BMI	0.705	<0.0001	
	panTG$^{1/2}$	-2.726	0.0063	
Model 3	Age	-0.401	0.0008	0.315
	Hip/waist	-29.583	0.0256	
	BMI	0.431	0.0452	
	Visceral fat$^{1/2}$	-1.322	0.333	

B: Multivariate analysis of AIR as dependent variable.

DI - disposition index, f/w% - fat/water%, BMI - body mass index, panTG - pancreatic triglyceride content, visceral fat - visceral fat area at level L2-L3, AIR – acute insulin response

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Altered microRNAs expression is associated with insulin resistance status in high fat diet fed mice
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Background and aims: Disturbances of microRNAs (miRs) expression or function play a role in several aspects of metabolism and glucose homeostasis. We have previously shown alterations in expression of miRs in insulin target tissues in the GK rat model of spontaneous type 2 diabetes, when com-

pared to normoglycaemic control strains. Investigations in our group in control mouse strains have demonstrated that high fat diet (HFD) feeding promotes the development of insulin resistance, obesity and fatty liver in 129S6 and C57BL6/J mice, whereas BALB/c are resistant to these experimentally induced phenotypes. The objective of the present work is to further study the implication of miRs in insulin resistance in two insulin target tissues (liver and adipose tissue) in these mouse strains.
Materials and methods: At five weeks, male mice of C57BL6/J, BALB/c and 129S6 were fed a normal carbohydrate diet (CHD) containing 5% fat, 19% protein, and 3.5% fibre or 40% HFD containing 32% lard oil and 8% corn oil ad libitum. At five months, liver and white adipose tissue were dissected (n=3-5), total RNA extracted and the expression of miR-125a, miR-27a, miR-222 and miR-29a were determined using ABI's taqman microRNA assays.
Results: 1/ When fed CHD, the expression of the four miRs was significantly higher in adipose tissue than in liver in the three mouse strains. Only miR-29a showed lower expression ($p<0.05$) in adipose tissue than in liver in 129S6 mice. 2/ When fed CHD, expression of miRs were highly variable between the 3 strains in both liver and adipose tissue. 3/ In HFD-fed 129S6 mice, expression of both miR-125a and miR-27a was upregulated in both liver (x4.6, $p<0.05$ and x2.1) and adipose tissue (x2.5, $p<0.05$ and x1.3). Tissue-specific miR over-expression was observed in liver for MiR-222 (x3.4, $p<0.05$) and in adipose tissue for miR-29a (x1.5). 4/ In HFD-fed C57BL6/J mice, miR-125a expression was not altered neither in liver nor adipose tissue. In response to HFD, expression of miR-27a was increased in liver (x2.4) and decreased in adipose tissue (-2.3). MiR-222 was over-expressed in liver (x3.4, $p<0.05$) and not altered in adipose tissue. Expression of miR-29a was unaffected in liver and decreased in adipose tissue (-1.6). 5/ In HFD-fed BALB/c mice, expression of miR-125a and miR-222 was not altered neither in liver nor adipose tissue. Expression of miR-29a was unchanged in liver and increased in adipose tissue (x1.4). Expression of miR-27a was unchanged in liver, but decreased in adipose tissue (-1.6).
Conclusion: Our results demonstrate the complex tissue- and strain-specific regulation of miRNA expression in experimentally induced insulin resistance, obesity and fatty liver disease. We observed a distinct correlation between miR altered expression and the metabolic status of HFD-fed mice. The 129S6 strain, very sensitive to HFD-induced glucose intolerance and obesity, exhibited miR over-expression in 75% of cases, whereas BALB/c, which is relatively resistant to HFD, showed miR altered expression in only 25% of cases. Our results provide strong evidence supporting a role of miR on impaired glucose homeostasis in models of spontaneous (GK) and experimentally induced (129S6) insulin resistance.
Supported by: Wellcome Trust and FGENTCARD

OP 11 Cardiovascular complications - experimental

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Adeno-associated-mediated nerve growth factor gene transfer prevents the development of heart microangiopathy and cardiomyopathy in mice
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Background and aims: Diabetes mellitus (DM) can cause cardiac dysfunction and heart failure independently of other risk factors like hypertension and myocardial infarction. The neurotrophin nerve growth factor (NGF) exerts cardioprotective effects but it is downregulated in the diabetic heart. The present study challenged the hypothesis that NGF gene transfer (GT) could prevent diabetes-induced cardiac dysfunction.
Materials and methods: Type-1 DM was induced in CD1 mice by streptozotocin injection (40 mg/Kg/day IP for five days). Two weeks later, GT with an adeno-associated vector serotype 2 carrying human *NGF* in the expression cassette (*AAV-2-hNGF*) or with an empty vector (*AAV-2-βGal*) was performed. Constructs were delivered into the left ventricle (LV) by 4 injections (total dose of $1x10^{11}$ pfu). Age-matched normoglycemic mice injected with *AAV-2-βGal* were used as controls.
Results: X-Gal staining confirmed successful GT of LV. Moreover, hNGF transgene expression was detected in plasma by ELISA at 12 weeks. Echocardiography (Visual Sonics) at 12 weeks after GT showed a deterioration of systolic function in diabetic mice (LV ejection fraction: 64.6±3.8 vs 73.1±6.9% in healthy controls; LV fractional shortening: 35.3±2.8 vs 42.5±6.3%; $P<0.05$ for both comparisons). In contrast, *AAV-2-hNGF* improved both LVEF and LVFS (113% and 121% respectively) as compared to diabetic controls ($P<0.05$ for both comparisons; N.S. vs healthy). Moreover, *AAV-2-hNGF* improved LV systolic pressure (LVSP) and contractility (expressed as dP/dt_{max} and dP/dt_{min}) measured by Millar catheter (LVSP: 76±4 vs 61±5 mmHg in diabetic controls; dP/dt_{max} and dP/dt_{min}: 141% and 149% of diabetic controls, respectively; $P<0.01$ for all comparisons). In addition, *AAV-2-hNGF* prevented enlargement of end-diastolic LV chamber volume (74±11 vs 89.1±13 µl in diabetic controls; $P<0.05$) and maintained the end-diastolic LV internal diameter (4.1±0.3 vs 4.4±0.3 µl in diabetic controls; $P<0.05$). Analyses in histological heart sections at 12 weeks indicated that diabetes induced microvessel rarefaction in the myocardium. By contrast, *AAV-2-hNGF* preserved capillary (measured as isolectin-B4 positive vessels) and small (diameter <50µm) arteriole (measured as isolectin-B4 and α-smooth muscle actin double positive vessels) densities in the hearts of diabetic mice (4255±222 vs 3440±155 capillaries/mm^2 in diabetic controls; $P=0.05$; 50±15.8 vs 37±13.9 arterioles/mm^2 in diabetic controls; $P=0.05$). Finally, as measured by fluorescent microspheres, DM reduced LV blood flow (0.39±0.1 vs 0.59±0.1 mL/min/g of tissue in healthy controls; P=NS), which was prevented by *AAV-2-hNGF* (0.69±0.1 mL/min/g of tissue; $P=0.05$ vs diabetic controls).
Conclusion: These results provide evidence that prolonged NGF overexpression prevents LV dysfunction and heart failure in the mouse diabetic heart and it also preserves cardiac microvasculature in the settings of diabetic cardiomyopathy.
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VEGF and CD36 are potential players in the crosstalk between perivascular fat and human smooth muscle cells
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Background and aims: The increase of fat mass in obesity is an important risk factor for the development of atherosclerosis. Specifically, perivascular fat in connection with obesity and the metabolic syndrome might be an activator of inflammation and dysfunction of the vessel wall. In previous studies we could show that adipocyte-conditioned media (CM) induced proliferation and migration of human smooth muscle cells with a concomitant increase in ICAM-1 expression. The combination (CMOA) of CM and oleic acid (OA) enhanced the proliferation in a synergistic way. CMOA induced

iNOS Expression, NO production and variable pro-inflammatory and proliferative signalling pathways. The aim of this study is to identify factors and mechanism, which are responsible for the effect of CM and the synergism with OA on the proliferation of smooth muscle cells.
Materials and methods: CM was generated from human *in vitro* differentiated human adipocytes and analysed for its content in adiponectin, IL-6 and VEGF. Proliferation of human coronary artery smooth muscle cells was measured via BrdU incorporation into DNA.
Results: The content of VEGF correlated significantly with the proliferative effect of CM (n=17, r=0.79, p=0.002) while adiponectin correlated negatively with CM-induced proliferation (n=22, r=-0.465, p=0.029). VEGF alone showed a 2.5-fold augmentation of proliferation and the combination of VEGF and OA (VEGFOA) act additive (5-fold). Incubation with CMOA induced a 2-fold increase of VEGF concentration compared with CM or OA alone, indicating that smooth muscle cells significantly contribute to proliferation by releasing VEGF for an autocrine/paracrine stimulation. Blocking VEGF with a specific antibody reduced the proliferative impact of CM, VEGF, OA, and VEGFOA significantly to control levels (CM=97 ±5 %, VEGF=106 ±5 %, OA=90±9 %, VEGFOA= 125±12 %). In contrast, VEGF-blocking was not sufficient to reduce CMOA-induced proliferation to control levels (from 569±40 % to 262±30 %). As CM and VEGF both significantly increase the expression of CD36 and as CM -treatment predisposes for increased lipid accumulation after OA addition, we propose that CD36 might be an additional player in CMOA-induced proliferation in smooth muscle cells. CD36 belongs to the family of scavenger receptors and is also identified as a fatty acid transporter. Moreover it has been speculated, that CD36 might play a pro-atherogenic role as CD36 interacts with VEGF and its receptor. Silencing of CD36 reduced the proliferative effect of OA and VEGF to control levels (OA= 118±19 %, VEGF= 80±6 %) while CM- and CMAO-induced proliferation remained significantly higher compared to control (CM=164±18 %, CMOA=257±30 %). Combining the silencing of CD36 with VEGF blocking, CMOA-induced proliferation could be reduced to only 150±7 % of the control level.
Conclusion: In this study we identified VEGF as an important factor for the proliferative effect of CM. The increase of CD36 expression by treatment with CM and VEGF could represent a key mechanism, how CM and OA synergistically increase proliferation and inflammatory signalling in smooth muscle cells. Further work is intended to elaborate how increased CD36 and lipid accumulation in smooth muscle cells are involved in the crosstalk between perivascular adipose tissue and the vessel wall.

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Increased expression of TIMP3 protects against diabetes and atherosclerosis

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Background and aims: Tissue inhibitor of Metalloproteinase 3 (TIMP3), an inhibitor of TNF-alpha Convertase (ADAM17) and other metalloproteinases is decreased in atherosclerotic plaque from patients with Type 2 diabetes and in tissues of humans and mice affected by obesity.
Materials and methods: Because obesity and atherosclerosis are associated with increased macrophage accumulation in different tissues, we used a transgenic approach under control of CD68 promoter to reconstitute TIMP3 levels to analyze the effects on metabolic and vascular homeostasis, using Diet Induced Obesity (DIO) to analyze the effect on insulin resistance and LDLR-/- background combined with atherogenic diet to analyze the effect on atherosclerosis. Transgenic (Tg) mice were identified by RT-PCR and confirmed by Southern blot. In Tg mice, TIMP3 overexpression was confirmed by RT-PCR, Western blot and Reverse Zymography in spleen, liver, peripheral blood monocytes and bone marrow derived macrophages, treated or not with LPS.
Results: In the DIO model, after 20 weeks of High Fat Diet (HFD), we observed that Tg mice compared with WT littermates showed a significant improved glucose tolerance and insulin sensitivity, measured by IPGTT and IPITT (p<0.01 for all, n=8 per group); a study of Fluoro-Deoxy-Glucose (FDG) uptake in combination with insulin infusion through Positron Emission Tomography scan (FDG-PET scan) revealed increased insulin induced glucose uptake in forelimbs of Tg mice compared with WT littermates (p<0.05, n=6 per group). Insulin, leptin, AST and ALT were lower and adiponectin increased in Tg mice compared with WT littermates (p<0.01 for all, n=8 per group). CT scan revealed significant reduction of visceral adipose tissue in Tg compared with WT littermates (p<0.05; n=3 per group). Histology of white adipose tissue (WAT), liver, kidney and aorta showed accumulation of lipids, intense fibrosis and increased inflammatory markers in WT compared with Tg mice. Tg revealed also significant reduction in adipose tissue vascularization (CD31 staining, p<0.05); however, mean adipocyte area was reduced in Tg compared with WT mice (p<0.01). Expression profiling for metabolic and inflammatory genes revealed that Tg compared with WT have significant higher levels for Adiponectin/CEBPalpha/PPARgamma/betaKLOTHO/FABP4/SOD1 (p<0.01 for all) and lower levels for MCP1/F4/80/SOCS3/Pref1/GP47/GP67 in WAT. In the liver TG showed increased Il-10 with decreased G6Pase/Socs3/GP47/GP67/Il-6 in liver (p<0.01 for all, n=5). Next we analyzed the effect of Timp3 overexpression in the LDLR-Tg and LDLR knockout mice fed Western Diet for 12 weeks. Histology of aortic roots from LDLR-Tg mice revealed 60% reduction in plaque surface (p<0.001), reduced F4/80, CD3 and nitrotyrosine staining (p<0.01 for all), increased collagen content and no necrotic cores, when compared to LDLR littermates (n=5 per group). Gene expression profiles in liver and aorta suggest reduced oxidative stress and less activation of inflammatory pathways in LDLR-Tg compared to LDLR mice.
Conclusion: Our data indicate that macrophage specific overexpression of TIMP3 protects from metabolic inflammation and related metabolic disorders such as insulin resistance, glucose intoleramce and atherosclerosis.
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Glucagon-like peptide-1 inhibits H2O2-dependent JNK phosphorylation and apoptosis in human cardiac progenitor cells

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Background and aims: The heart possesses a compartment of multipotent cardiac progenitor cells (CPCs), which provides a constant tissue renewal. Increased CPC apoptosis has been proposed as a mechanism of myocardial dysfunction, leading to heart failure. Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by the small intestine in response to nutrient ingestion. There is growing evidence suggesting that GLP-1 may regulate cardiac function and promote survival of cardiac cells in environmental stress conditions. The aim of this study was to investigate the protective effects of GLP-1 on H2O2-induced apoptosis in CPCs isolated from adult human heart biopsies, obtained from the auricle in the course of open heart surgery.
Materials and methods: Biopsy-obtained cells showed typical features of mesenchymal multipotent cells, including the ability to proliferate up to the 15th passage, and the differentiation potential toward the osteogenic and chondrogenic lineages. Analysis of CD105, c-kit, CD34, CD31, CD133, and CD45 by flow cytometry, and lineage-specific intracellular markers by quantitative real-time PCR, including GATA-4 and MEF-2C, confirmed that the isolated cells represent resident cardiac progenitors. Alpha-MHC transcripts, which characterize differentiated cardiac cells, were undetectable. Expression and activation levels of the proteins under investigation were evaluated by immunoblotting techniques.
Results: Exposure of CPCs to 0.5 mM H2O2 for 20-120 min induced a 2-fold increase in cell apoptosis, measured by evaluation of cytosolic oligosomes (p<0.05) and cleaved caspase-3 (p<0.05). Exposure to H2O2 induced a 3-fold increase in the phosphorylation of Erk1/2 (p<0.05), a 1.5-fold increase in the phosphorylation of JNK1/2 (p<0.05) and a 6-fold increase in the phosphorylation of Akt (p<0.05). When JNK activation was inhibited using the specific inhibitor SP600125 (10 μM), H2O2-dependent JNK phosphorylation appeared to be prevented, and H2O2-induced apoptosis was decreased by 40% (P<0.05). SP600125 also inhibited the phosphorylation of the JNK substrate c-jun, but had no effects on the JNK upstream kinases MKK-4 and MKK-7. Preincubation of CPCs with 20 nM GLP-1 for 16 h inhibited both H2O2-induced activation of JNK 1/2 (p<0.05 vs. H2O2-treated CPCs) and apoptosis (p<0.05 vs. H2O2-treated CPCs). GLP-1 incubation had no evident effect on Akt and Erk1/2 phosphorylation in response to H2O2. However, exposure of CPCs to GLP-1 (10-20 nM) was associated with a significant increase in the phosphorylation of the PKA-dependent transcription factor CREB.
Conclusion: Thus, GLP-1 prevents H2O2-mediated apoptosis in human CPCs, at least in part by interfering with the activation of JNK. A direct pro-survival mechanism in myocardial progenitor cells may contribute to the observed protective effects of GLP-1 on the heart.
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Octyl-D-carnosine attenuates atherosclerosis and renal disease in ApoE null mice fed a western diet

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Background and aims: Reactive carbonyl species generated by oxidation of endoperoxides in lipoproteins react with proteins to form advanced lipoxidation endproducts (ALEs), which have been implicated in both atherosclerosis and renal disease. L-carnosine was shown to act as a quencher of reactive carbonyl species, but, in humans, it is rapidly inactivated by carnosinase. This study evaluated the effect of carnosinase-resistant octyl D-carnosine (ODC) on the development of atherosclerosis and renal disease in the apoE null mouse model.
Materials and methods: Adult female ApoE null mice, fed either a western, pro-atherogenic, high fat diet (HFD, 42% fat, 0.2% cholesterol) or a standard, normal fat diet (NFD), were treated with ODC (Flamma SpA, Chignolo d'Isola, BG, Italy; 60 mg/kg body weight in the drinking water) or vehicle for 12 weeks. Aortic and kidney lesions were evaluated, together with expression of inflammatory and disease progression markers.
Results: ODC-treated HFD-fed mice showed significantly reduced lesion area at the level of the aortic sinus (434.9±49.9 vs. 527.9±49.6 μm x 10^{-3}, P<0.01) and, particularly, of the brachiocephalic artery (22.3±4.4 vs. 43.3±6.5 % of luminal occlusion, P<0.01), as compared with untreated animals. Treatment also produced a more stable plaque phenotype, with less foam cell accumulation, inflammation and apoptosis and increased clearance of apoptotic bodies, resulting in reduced necrotic core formation (11.8±3.8 vs. 25.3±5.4%, P<0.001). Fibrosis was increased (42.6±5.8 vs. 28.7±7.8 % of lesion area, P<0.01) and disruption of vessel wall architecture was reduced, with less elastin degradation and pseudo-microaneurysm formation. Oil red O staining of *en-face* aorta preparations showed a significantly reduced lipid accumulation in ODC-treated vs. untreated HFD-fed ApoE null mice (13.2±2.5 vs. 19.2±2.0%, P<0.001). Increases of protein content of F4/80, and CXCR3 as well as of mRNA expression of F4/80, CXCR3, VCAM-1, MCP-1, TNF-α, IFN-γ, MMP-9, and CHOP were significantly lower (or even normalized) and those of anti-inflammatory IL-4 and Il-10 significantly increased in ODC-treated versus untreated HFD-fed mice. This was associated with reduced tissue content of HNE adducts, oxLDLs, and nitrotyrosine and mRNA expression of the ALE-receptors CD36, TLR-2 and 4, RAGE and galectin-3 in HFD-fed ODC-treated versus untreated ApoE null mice. Likewise, renal lesions were significantly attenuated in ODC-treated versus untreated HFD-fed mice on HFD, with lower foam cell accumulation, inflammation, apoptosis, glomerular and tubulo-interstitial fibrosis, content of HNE adducts, oxLDLs, and nitrotyrosine and expression of pro-inflammatory and pro-fibrotic mediators. Serum ALE, pentosidine, carbonylated proteins, and isoprostane-8-epi-$PGF_{2\alpha}$ levels were also lower in ODC-treated vs. untreated HFD-fed ApoE null mice.
Conclusion: These data support a central role for lipoxidation in atherosclerosis and renal disease and indicate that quenching of carbonyl adducts with a D-carnosine ester may represent a promising approach to the prevention and treatment of these disorders.

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Methylglyoxal-mediated cardiovascular cell dysfunction: role of poly (ADP-ribose) polymerase

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Background and aims: Methylglyoxal (MGO), a glycolysis derived reactive dicarbonyl compound, has been implicated in cardiovascular complications of diabetes. MGO reacts with many short and long-lived cellular proteins disrupting their synthesis and function which may lead to increased cellular oxidative stress. Overactivation of the DNA repair enzyme poly (ADP-ribose) polymerase (PARP) following oxidative stress-induced DNA damage with subsequent depletion of cellular high energy phosphate and NAD levels has been implicated in endothelial cell dysfunction in diabetes. The aims of this study were twofold, firstly to determine whether MGO-mediated activation of PARP was responsible for the observed cardiovascular cell dysfunction and hence the link between hyperglycaemia and PARP activation already reported in cardiovascular cells. Secondly, to determine whether the cardioprotective agent, resveratrol could protect against MGO-mediated dysfunction.
Materials and methods: The effects of MGO on acetylcholine-mediated NO dependent vasorelaxation were tested using ex vivo rat aortic rings exposed to 0.1, 0.3 and 1 mM MGO for 1, 2 and 4h. The role of PARP activation in MGO-mediated dysfunction was evaluated by the co-administration of the specific PARP inhibitor PJ34 (10 μM) with MGO (0.3 mM). The effect of the cardiovascular protective agent resveratrol (3 μM) on MGO-mediated cardiovascular cell dysfunction was also determined. MGO effects on cardiac H9c2 myocyte cell viability was measured by MTT assay and oxidative stress was measured using the NBT assay.
Results: MGO dose and time dependently caused endothelial cell dysfunction, 0.1 mM MGO had no effect on endothelial cell-mediated vascular relaxation following 1, 2 or 4h exposure, whereas 0.3 and 1 mM though having no effect at 1h exposure, caused significant endothelial cell dysfunction at both 2 and 4h. Exposure of aortic rings for 2h to MGO (0.3 mM) increased the IC50 for acetylcholine-mediated relaxation from 15±3 nM to 82±4nM (p<0.01), no further damage was seen either with 1 mM MGO or an increased exposure time (4h). Simultaneous exposure of aortic rings to MGO (0.3 mM) and the PARP inhibitor PJ34 (10 μM) for 2h protected against the MGO-mediated dysfunction significantly reducing the acetylcholine IC50 from 81±5 nM to 18±4 nM (p<0.01 vs. MGO alone), also simultaneous application of resveratrol (3 μM) also protected endothelial cell function reducing the IC50 to 19±6 nM (p<0.01 vs. MGO alone). MGO dose dependently reduced H9c2 myocyte cell viability following 24h exposure. Simultaneous addition of resveratrol increased H9c2 cell viability from 28±0.5% with 0.8 mM MGO alone to 36±2%, 51±2% and 54±1% with 1, 3 and 10 μM respectively (p<0.05 vs. MGO alone). Exposure of H9c2 cells to MGO for either 4 or 6h significantly increased cellular oxidative stress. MGO at 1 mM increased oxidative stress by 25±11% and 46±12% above untreated cells following 4 and 6h exposure respectively (p<0.05 vs. untreated cells).
Conclusion: MGO exposure causes both endothelial and myocyte cellular dysfuction, effects which appear to be mediated by increased cellular oxidative stress and activation of PARP. Resveratrol also appears to confer a protective effect against MGO-mediated endothelial and myocyte cellular dysfunction.

OP 12 Metabolic control of beta cells

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Is G6PC2/IGRP a component of the beta cell glucose sensor?

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Background and aims: Glucose-6-phosphatase catalyzes the hydrolysis of glucose-6-phosphate (G6P) to glucose and inorganic phosphate. The glucose-6-phosphatase catalytic subunit (G6PC) gene family comprises three members, G6PC, G6PC2 and G6PC3. G6PC, also known as G6Pase, is predominantly expressed in liver and kidney where it catalyses the terminal step in the gluconeogenic and glycogenolytic pathways. G6PC2, previously known as the islet-specific glucose-6-phosphatase catalytic subunit related protein (IGRP), is selectively expressed in insulin producing beta cells. In vitro, G6PC2 hydrolyzes G6P at a much lower rate than G6PC raising the question as to whether G6P is a physiologically important substrate for this protein.
Materials and methods: To assess the physiological importance of G6pc2, we have generated G6pc2-null mice, backcrossed onto a C57BL/6J background, and performed a phenotypic analysis focusing primarily on energy metabolism and pancreatic hormone secretion.
Results: 16 week old G6pc2 KO mice on a chow fed diet exhibit no differences in body weight and no gross anatomical or behavioral changes. However, following a 6 hour fast, a decrease in blood glucose was observed in both male (WT: 130.7+/-3.6; KO: 109.2+/-3.5; $p<0.001$) and female (WT: 121.9+/-4.3; KO: 104.3+/-4.4; $p<0.01$) G6pc2 KO mice relative to wild type (WT) littermates, while plasma insulin and glucagon concentrations were unchanged. Because glycolytic flux has been shown to determine the S0.5 of glucose-stimulated insulin secretion (GSIS) these observations are consistent with a model in which the glucose-6-phosphatase activity of G6PC2 opposes the action of glucokinase and thereby modulates the S0.5 of GSIS. Deletion of the G6pc2 gene is therefore predicted to abolish glucose cycling, increase glycolytic flux and lower the S0.5 of GSIS. Additional studies were performed to explore this concept. Pancreas perfusion experiments in which islets were challenged with variable glucose concentrations demonstrated that deletion of G6pc2 results in a leftward shift in the S0.5 of GSIS. A similar shift was observed in islet perifusion experiments. In addition, following static incubation of islets in a sub-maximal 11 mM stimulatory glucose concentration, islets isolated from G6pc2 KO mice displayed enhanced GSIS relative to islets isolated from WT mice (WT: 0.899 (%content/30 min)+/-0.142; KO: 2.116+/-0.204; $p<0.01$). Finally, intraperitoneal glucose tolerance tests, again using a submaximal stimulatory glucose concentration, demonstrate that G6pc2 KO mice do not have enhanced glucose tolerance but rather lower blood glucose at all time points, a result that is again consistent with G6pc2 regulating the S0.5 of GSIS.
Conclusion: The existing dogma in the islet field proposes that glucokinase is the beta cell glucose sensor. The significance of our observations is that they challenge this dogma and suggest that G6PC2 is a fundamental inhibitory component of that sensor. Instead we propose that a glucokinase/G6PC2 futile cycle acts as the beta cell glucose sensor determining glycolytic flux and hence the S0.5 of GSIS. This conclusion is consistent with genome wide association studies that have recently shown that single nucleotide polymorphisms in the G6PC2 gene are associated with variations in fasting plasma glucose levels in humans.
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Identification of an intracellular metabolic signature impairing beta cell function

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Background and aims: Chronic hyperglycaemia promotes the progressive failure of pancreatic β-cells in patients with diabetes mellitus, a clinically highly relevant phenomenon known as glucotoxicity. Assuming that changes of the intracellular metabolism contribute to this progressive β-cell failure, an unbiased metabolite profiling analysis was carried out to identify the metabolic signature of β-cells during high glucose exposure.
Material and methods: Isolated human islets and INS-1E cells were cultured at 5.5 to 33.3 mM glucose from 1 to 96 h with or without 6-aminonicotinamide (6-AN), a potent inhibitor of 6-phosphogluconic acid dehydrogenase. After the glucose pre-treatment, islets underwent an additional glucose challenge at 5.5 and 22.2 mM glucose and of p-ERK, glucose stimulated insulin secretion (GSIS), insulin mRNA were analysed. Metabolites were analysed in islet lysates on a Leco Pegasus 3 time-of-flight mass spectrometer (Leco). PCA was performed with MATLAB 7.0 (Mathworks).
Results: To identify potential novel mediators of insulin gene suppression by chronic high glucose treatment, an unbiased time course analysis of metabolites was performed in the β-cells INS-1E and in human islets, during which 73 metabolites were uniquely identified. Principal component analysis (PCA) revealed that prolonged exposure to high glucose caused clear differentiation compared to early time points thus demonstrating the differential impact of acute and chronic glucose exposure on the β-cell. The most important metabolites driving the separation from acute to chronic glucose were gluconic acid, glucono-delta-lactone and 6-phosphogluconic acid from the pentose phosphate pathway. The addition of 6-AN at low glucose, promoted the accumulation of the pentose phosphate metabolites thus partially reflecting the metabolic state of the β-cells under high glucose. Analysis of insulin mRNA revealed that 6-AN and the accompanied rise in pentose phosphate metabolites lead to a 3.5-fold decrease of insulin gene expression compared to low glucose alone, similarly as exposure to chronic high glucose for 3 days (3.3-fold decrease). Glucose-dependent regulation of insulin transcription is dependent on extracellular signal-regulated protein kinases (ERK1/2). ERK1/2 kinase is activated by acute glucose exposure. In contracts, we show here that pre-incubation of elevated glucose completely abolished a further glucose mediated ERK induction, which correlated with the loss of GSIS, suggesting that ERK stimulation, is essential for GSIS. Accumulation of pentose phosphate pathway metabolites in islets by 6-AN at low glucose concentrations, was able to promote ERK1/2 phosphorylation to a similar extent as long-term high glucose treatment, but also failed to maintain GSIS.
Conclusions: Based on unbiased metabolite analyses, the here presented insights provide novel perspectives in the therapeutic goal to preserve and potentially improve beta-cell function in patients with diabetes mellitus.

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miR-29a, miR-29b and miR-124 contribute to pancreatic beta cell specific silencing of monocarboxylate transporter 1 (*Mct1/Slc16a1*)

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Background and aims: Glucose metabolism in pancreatic beta cells is specialised to efficiently couple glucose oxidation to ATP production, critical for stimulating insulin secretion. Alternative metabolic pathways that could interfere with glucose sensing are suppressed by specifically "disallowing" expression of certain genes in beta cells. For example, *MCT1* (*SLC16A1*) encodes a plasma membrane monocarboxylate (pyruvate/lactate) transporter which is widely expressed in other tissues but not in beta cells. The effects of inappropriate expression of *MCT1* are shown in the rare genetic disorder, Exercise Induced HyperInsulinism, linked to mutations in the *MCT1* promoter. During strenuous physical exercise, the presence of the transporter in affected individuals is presumed to allow circulating pyruvate to enter beta cells, resulting in inappropriate insulin release and consequent hypoglycaemia. We aimed here to identify the mechanisms by which expression of *MCT1* is specifically disallowed in beta cells. The *MCT1* promoter drives low but significant reporter gene expression in the MIN6 beta cell line suggesting that additional post-transcriptional mechanisms may be responsible for silencing this gene. We therefore investigated whether microRNAs (miRNA) expressed in beta cells contribute to the tissue specific silencing of this gene.
Materials and methods: Publicly available high throughput sequencing data from beta cells and thirteen other mouse tissues were interrogated to determine the abundance and specificity of microRNA expression. Potential miRNA binding sites within the *MCT1* 3′ UTR were identified using miRanda and PicTar algorithms. Hits were validated by luciferase assay on both human and mouse *MCT1* 3′ UTRs, and precise locations of binding sites confirmed by site-directed mutagenesis. Effects on endogenous *Mct1* expression were determined by stable expression of miRNAs in the hepatocyte-derived mhAT3-F cell line where *Mct1* mRNA is abundant.

Results: Bioinformatic analysis of miRNA expression in beta cells ranked all detected miRNAs by a combined abundance and specificity score, successfully identifying known beta cell specific miR-375 and miR-7 within the top three. Eight miRNAs were predicted to target the *Mct1* 3′ UTR, of which miR-29a, miR-29b and miR-124 produced large and significant decreases in expression by luciferase assay in HEK293 cells. All three miRNAs fall within the top third of miRNAs ranked by combined beta cell abundance and specificity score. Mutating the identified binding sites abolished the effect of miR-124 and miR-29b and significantly reduced the effect of miR29a by luciferase assay. Stable expression of miR-29a and miR-124 in mhAT3-F cells virtually abolished, and miR-29b considerably reduced expression of Mct1 protein. Mutating either binding site significantly increased expression of luciferase bearing the *Mct1* 3′ UTR in MIN6 beta cells, confirming these miRNAs are expressed at sufficient levels to downregulate *Mct1*.
Conclusion: Our interrogation of publicly available miRNA expression data provides a novel prediction of miRNAs important for beta cell function. Among these we identify miR-29a, miR-29b and miR-124 as capable of downregulating *Mct1* through a direct interaction with cognate binding sites in the 3′ UTR. We propose that the coordinated action of these miRNAs contributes to the beta cell specific silencing of *Mct1* required for efficient glucose sensing.
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Inducible expression of Monocarboxylate Transporter 1 (Mct1/Slc16a1) in the beta cell of transgenic mice: a model of exercise induced hyperinsulinism
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Background and aims: The plasma membrane monocarboxylate (pyruvate/lactate) transporter MCT1/SLC16A1 is expressed at vanishingly low levels in pancreatic beta cells but at high levels in other tissues. The absence of this transporter may serve a dual function. Firstly, it may prevent circulating pyruvate from entering the beta cell and inappropriately stimulating insulin secretion. In the rare dominant genetic disorder Exercise Induced HyperInsulinism (EIHI), linked to mutations in the *MCT1* promoter, pyruvate produced by muscle during vigorous physical exercise stimulates insulin secretion, resulting in hypoglycaemia. Secondly, by preventing loss of pyruvate generated by glycolysis, it may ensure that glucose is efficiently oxidised by mitochondria, generating ATP to trigger insulin secretion. While the mutations associated with EIHI increase expression of reporter genes in beta cell lines and patients exhibit increased MCT1 expression in fibroblasts, there is no direct evidence that MCT1 is over-expressed in patient beta cells. Here we have created a transgenic mouse model in which MCT1 is expressed in pancreatic beta cells and examine the impact on pyruvate or glucose tolerance *in vivo*.
Materials and methods: We established a transgenic mouse line in which a tetracycline-regulatable promoter controls expression of human *MCT1*, then crossed this with mice expressing the reverse-tetracycline transactivator (rtTA) under control of the rat insulin promoter. This permits beta cell-specific expression of *MCT1* upon addition of doxycycline only in mice bearing both transgenes. Littermates containing no transgenes were used as controls. *MCT1* expression was induced by administering doxycycline (1 g/l) in drinking water for at least 7 days. Glucose (1 g/kg body weight) or pyruvate (0.5 g/kg) were administered intraperitonealy following 16 hour fast, and blood glucose levels were measured.
Results: Following induction of *MCT1* expression, double transgenic (DT) mice showed a non-significant trend to lower fasting blood glucose relative to wildtype littermate controls (WT). Fasting blood glucose ± SEM: DT = 5.17 ± 0.34 mmol/l, n=13; WT = 6.24 ± 0.33 mmol/l, n=5; p= 0.091. In the absence of doxycycline there was no difference in the effect of pyruvate on blood glucose in DT v WT. Blood glucose 30 min after pyruvate challenge ± SEM: DT = 10.37 ± 0.45 mmol/l, n=13; WT = 9.90 ± 0.58, n=5; p=0.573). However, following doxycycline induction DT mice had significantly lower blood glucose: DT = 7.18 ± 0.40 mmol/l, n=13; WT = 8.78 ± 0.51 mmol/l, n=5; p=0.039. Thus, insulin secretion from beta cells appears to become sensitive to pyruvate in DT mice, lowering blood glucose. Interestingly, these mice also showed decreased glucose tolerance. Blood glucose 30 min after glucose challenge ± SEM: DT = 18.01 ± 0.87 mmol/l, n=11; WT = 13.16 ± 0.48 mmol/l, n=5; p=0.003). This is consistent with loss of pyruvate from beta cells lowering the yield of ATP from glucose metabolism hence reducing insulin secretion.
Conclusion: Expression of MCT1 in beta cells is sufficient to replicate the key feature of EIHI, as pyruvate challenge lowers blood glucose relative to controls. The demonstration that DT mice are also glucose intolerant reveals the importance of suppressed MCT1 expression in beta cells for normal glucose stimulated insulin secretion. Activating mutations in the *MCT1/SLC16A1* gene might also, therefore, increase susceptibility to type 2 diabetes.
Supported by: Wellcome Trust

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Regulation of pyruvate dehydrogenase by phosphorylation in the pancreatic beta cell
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Background and aims: In the pancreatic beta-cell, glycolysis-derived pyruvate is taken up into the mitochondria where it is oxidized by pyruvate dehydrogenase (PDH; oxidative pathway) or carboxylated by pyruvate carboxylase, providing carbons to the TCA cycle (anaplerotic pathway). Recent studies suggest an important role of the anaplerotic pathway in glucose-stimulated insulin secretion. The relative flux through the two pathways in beta-cells may in part be determined by the PDH activity, which is regulated by phosphorylation/dephosphorylation of the E1 alpha subunit. Phosphorylation by PDH-kinases (PDK) leads to inactivation of PDH, while removal of phosphate by the calcium activated PDH-phosphatases (PDP) stimulates PDH activity. Here we have studied regulation of PDH by phosphorylation during glucose stimulation of the pancreatic beta-cell.
Materials and methods: INS-1E cells and rat islets were exposed to glucose or alternative secretagogues in the presence or absence of calcium signals. PDH phosphorylation was followed by Western-blotting, using an antibody specifically recognizing the phosphorylated (Serine-293) PDH E1 alpha subunit. The blots were then re-probed with an antibody for detection of total PDH E1 alpha protein. Expression of PDK isoforms (PDK1, PDK2, PDK3 and PDK4) in INS-1E cells was analyzed by RT-qPCR.
Results: Glucose stimulation leads to increase in mitochondrial calcium and thus would be expected to stimulate PDP resulting in dephosphorylation of PDH. Surprisingly, high glucose (16.7mM) or leucine (20mM) caused PDH E1 alpha phosphorylation in INS-1E cells and rat islets. A significant increase in phosphorylation was already observed after 5 minutes of glucose stimulation. This effect was also seen under conditions of blocked insulin secretion, ruling out the possibility that increase in PDH E1 alpha phosphorylation is a consequence of insulin receptor signaling. Preventing calcium signals during glucose stimulation further increased PDH E1 alpha phosphorylation while raising calcium by KCl (30mM) decreased phosphorylation. These findings are consistent with the known regulation of PDP activity by calcium. Under resting glucose (2.5mM) augmenting or depleting calcium did not affect PDH E1 alpha phosphorylation. This shows that nutrient stimulation and not calcium per se is the principal signal for PDH phosphorylation. We hypothesized that during glucose stimulation total PDK activity is more activated than the PDP activity leading to an enhanced PDH phosphorylation despite the glucose-stimulated mitochondrial calcium rise. We proceeded to determine the kinase (or kinases) responsible for the observed PDH E1 alpha phosphorylation. Expression of PDK1, PDK2 and PDK3 was detected in INS-1E cells. PDK4 was not detectable in this cell type. Remarkably, PDK3 transcript level was significantly higher than in any of the tested tissues, suggesting that this isoform may be particularly important in insulin-secreting cells.
Conclusion: PDH is rapidly phosphorylated following glucose stimulation in INS-1E cells and rat islets. Nutrient secretagogues regulate PDH phosphorylation and thereby determine to what extent the oxidative and anaplerotic pathways are utilized. Inactivation of PDH by phosphorylation should favor anaplerosis, which is required for the export of mitochondrial metabolites and may thereby affect the formation of mitochondria-derived coupling factors.
Supported by: Swiss National Science Foundation

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Glucose induces oscillations of the ATP/ADP ratio in individual beta cells
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Background and aims: The ATP/ADP ratio has a central messenger function in β-cells, linking changes in glucose metabolism to electrical activity,

Ca^{2+} signaling and insulin secretion. Biochemical and electrophysiological analyses as well as single-cell and islet recordings of e.g. oxygen consumption, NAD(P)H fluorescence and mitochondrial membrane potential have provided evidence for metabolic oscillations in β-cells, but it has been difficult to directly demonstrate fluctuations of the ATP/ADP ratio. Application of the firefly luciferase bioluminescence assay in single β-cells is very challenging and has provided limited information about ATP rather than the ATP/ADP ratio. Using a novel fluorescence protein-based ATP/ADP ratio sensor, the aim of the present study was to monitor changes in ATP/ADP ratio in single glucose-stimulated β-cells.
Materials and methods: Circularly permuted fluorescent protein cpmVenus inserted into the T-loop of the ATP-binding bacterial protein GlnK1 (Perceval) was used as a cytoplasmic biosensor for ATP/ADP ratio in individual MIN6 β-cells. In some experiments the red fluorescent protein tdimer2 was used as a reference. Changes of cytoplasmic fluorescence were recorded with confocal microscopy.
Results: MIN6 β-cells expressed Perceval with essentially even distribution in the cytoplasm. The ATP-sensitivity of the probe was investigated after permeabilization of the plasma membrane with α-toxin. Changes of the medium concentration of ATP from 0 to 1, 3 and 10 mM resulted in concentration-dependent increase of Perceval fluorescence with half-maximal effect at about 3 mM ATP (n=9). Most intact cells showed stable fluorescence in the presence of 3 mM glucose. Application of 5 μM of the mitochondrial uncoupler FCCP dramatically diminished the Perceval fluorescence. Elevation of the glucose concentration from 3 to 20 mM induced oscillations of Perceval fluorescence with a frequency of 0.24±0.01 min^{-1} in >95% of the cells (n=107). In most cells (71%) the oscillations originated from an elevated level and the amplitude averaged 15±1% of the fluorescence in 3 mM glucose. The increase in ATP/ADP ratio was counteracted by rise of cytoplasmic Ca^{2+}, since hyperpolarization with the K_{ATP} channel activator diazoxide (250 μM) increased the Perceval fluorescence with maintenance of the glucose-induced oscillations (50%) or stable elevation. Moreover, membrane depolarization with 30 mM K^+ in the presence of 3 mM glucose caused pronounced decrease in Perceval fluorescence.
Conclusions: Glucose stimulation of MIN6 β-cells triggers oscillations of the cytoplasmic ATP/ADP ratio. Cytoplasmic Ca^{2+} negatively regulates the ATP/ADP ratio, but metabolic oscillations are maintained the absence of stimulated entry of Ca^{2+}.

OP 13 Incretin based therapies: new developments

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Exenatide added to insulin glargine-treated patients with type 2 diabetes provided excellent fasting and postprandial control with weight loss and no increased risk of hypoglycaemia

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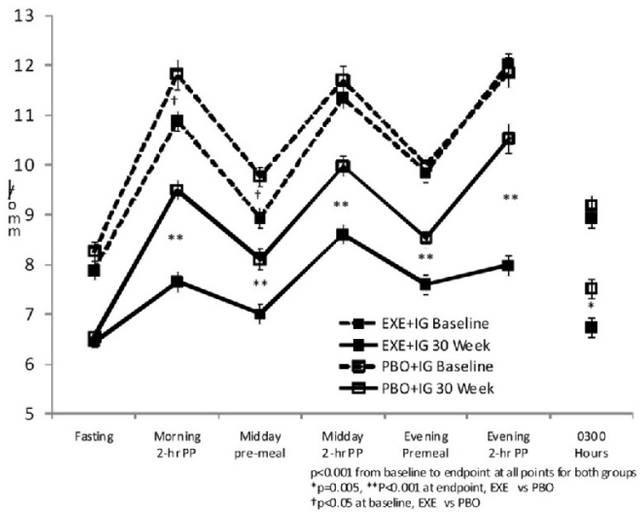
Background and aims: This is the first double-blind, placebo-controlled study adding exenatide (EXE) treatment in patients with type 2 diabetes suboptimally controlled (A1C ≥7.1 ≤10.5%) with basal insulin glargine therapy ± oral agents. The primary efficacy variable was change in A1C from baseline to 30 weeks. Prandial glycemic control measures included 7-point self-monitored blood glucose (SMBG) values, continuous glucose monitoring (CGM) data, and 1,5-anhydroglucitol (1,5-AG), a marker that is inversely proportional to average glycemia with greater sensitivity to postprandial (PP) excursion.
Materials and methods: A total of 259 patients (mean age 59 years, weight 94.4 kg, A1C 8.41%, diabetes duration 12.3 years, and insulin dose 48 U [0.51 U/kg]) were randomized to add either exenatide (10μg BID, n=137) or placebo (n=122) to insulin glargine therapy. Groups were generally comparable at baseline. At randomization, insulin dose was maintained (A1C >8.0%) or decreased by 20% (A1C ≤8.0%) for 5 weeks and then titrated to achieve a target fasting glucose of <5.6 mmol/L.
Results: At 30 weeks, A1C decreased by -1.71% to 6.70% with EXE treatment, and by -1.00% to 7.41% with placebo (PBO [p<0.001]). Endpoint fasting glucose values were not different between treatment groups (EXE 6.5±0.1, PBO 6.6±0.1 mmol/L, p=0.633). All non-fasting SMBG values were significantly lower for EXE (Figure). The 24-hour average glucose measurement from CGM (n=23) decreased in EXE by -3.2 to 6.6±0.4 mmol/L, and in PBO by -1.9 to 8.0±0.4 mmol/L (p=0.039). While baseline values of 1,5-AG were comparable between EXE and PBO (7.0±0.42 and 5.8±0.44 μmol/L, p=0.056), endpoint values were significantly higher for EXE than for PBO (12.7±0.5 vs 10.6±0.6 μmol/L, p=0.003). Weight decreased in EXE (-1.8±0.3kg, p<0.001) and increased in PBO (+1.0±0.3kg, p=0.011). Insulin dose increased more in PBO (20±2 U, p<0.001) than in EXE (13±2 U, p<0.001), (p=0.026 between groups). Minor hypoglycemia was similar for EXE and PBO (rate: 1.43±0.31 and 1.24±0.30 episodes/patient/year, p=0.666); major hypoglycemia (nocturnal) occurred twice in one PBO-treated patient. Adverse events were significantly greater for EXE vs PBO : nausea (41 vs 8%), diarrhea (18 vs 8%), vomiting (18 vs 4%), headache (14 vs 4%), and constipation (10 vs 2%).
Conclusion: To our knowledge, this is the first report of a controlled trial using a GLP-1-receptor agonist with basal insulin. Addition of EXE therapy in patients treated with insulin glargine improved A1C by contributing a prandial effect with no increased risk of hypoglycemia.



Supported by: Eli Lilly and Company and Amylin Pharmaceuticals

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Response at 3 months to insulin dose decisions made at exenatide initiation in the Association of British Clinical Diabetologists (ABCD) nationwide exenatide audit

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Background and aims: To learn from experience of exenatide in real clinical use in the UK, ABCD began a nationwide audit in December 2008. Though exenatide is not licensed for use with insulin many contributors to the audit used the combination. There is uncertainty about what should be done with insulin dose when exenatide is added. We therefore studied the response at 3 months after exenatide initiation in relation to insulin dose decisions made when exenatide was started.
Materials and methods: Patients were analysed according to three groups at initiation: non-insulin users (Group 1), insulin users in whom insulin was stopped (Group 2), and insulin users with insulin continued (Group 3). Group 3 was divided further into groups who had insulin doses unchanged, reduced by 1-40% (mean dose reduction 25.3%), 41-60% (mean 49.7%) and 61-99% (mean 67.4%) for further analyses. HbA1c and weight changes were compared within and across groups at baseline and 3 months. Correlation between insulin dose reduction and HbA1c and weight changes were assessed. Differences in group characteristics were examined.
Results: Amongst 6717 patients in the audit, exact data on diabetes treatment at baseline and initiation as well as suitable HbA1c and weight data were available for 2575 and 2454 patients respectively. The distribution in each group (HbA1c data, weight data) was: Group 1 (1626, 1545), Group 2 (275, 271) and Group 3 (674, 638). Mean age (54.1, 54.8, 55.0yrs), BMI (40.0, 39.3, 40.3kg/m^2), and initial HbA1c (9.5, 9.7, 9.6%) were not statistically different between groups. Group 2 had lower baseline weight than Group 1 (110.8 v 114.6kg, p=0.009). Group 2 had longer diabetes duration than Group 1 (10.8 v 8.3yrs, p<0.001), but lesser duration (10.8 v 12.3yrs, p<0.001) and total insulin dose (92 v 121U, p<0.001) compared with Group 3. Group 1 and 3 achieved significant HbA1c reductions (-0.95%, -0.53%, both p<0.001), but not Group 2 (-0.01%, p=0.948). 48% in Group 2 had HbA1c increases, with 15% ≥ 2.0%. All 3 groups achieved weight reductions (-3.7, -6.6 and -4.3kg, all p<0.001). Across groups, Group 2 achieved no HbA1c benefit compared with Group 1 and 3 (both p<0.001) but the most weight benefit (both p<0.001). Group 3 had intermediate results showing less HbA1c reduction but more weight loss than Group 1 (p<0.001, p=0.007 respectively). Among Group 3, weight reduction, but not HbA1c change, correlated with total insulin dose reduction (p=0.003, p=0.158 respectively). Subgroup analyses revealed the group with average insulin dose reduction of around half, and two-thirds, achieved more weight loss than the group with no dose changes (p=0.010, and p=0.037) or average dose reduction of a quarter (p=0.018, and p=0.035 respectively). Analyses on HbA1c changes among different insulin dose reduction groups did not reveal any significant differences. No severe hypoglycaemia was recorded in Group 3.
Conclusion: Our analysis shows that continuing insulin at exenatide initiation was safe and yielded HbA1c and weight reductions. Progressive insulin dose reductions yielded increasing weight loss but at the expense of HbA1c reduction, although there was no clear threshold when glycaemic reduction was negatively affected. When insulin was substituted, rather than combined with exenatide, almost half of patients had worsened glycaemic control.
Independent audit supported by an unrestricted grant from Eli Lilly Ltd

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Superior glycaemic control with taspoglutide, a once-weekly human GLP-1 analogue, compared with twice daily exenatide in type 2 diabetes: the T-emerge 2 Trial

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Background and aims: Taspoglutide is a once-weekly (QW) human GLP-1 analog in Phase 3 development. T-emerge 2 compared the safety and efficacy of QW taspoglutide (Taspo) with twice-daily (BID) exenatide (Exe) in adult patients with type 2 diabetes (T2DM) inadequately controlled with metformin+/-thiazolidinedione.
Materials and methods: In this multinational, open-label study, 1149 subjects were randomized (1:1:1) to subcutaneous Taspo 10 mg QW (Taspo10), Taspo 10 mg QW for 4 wks titrated to 20 mg QW (Taspo20), or Exe 5 mcg BID for 4 wks titrated to 10 mcg BID. Primary outcome was HbA1c change at wk 24, testing for non-inferiority (NI) vs Exe (using 2-sided 95% CI for HbA1c difference and noninferiority margin of 0.4%) in the intent-to-treat (ITT) population. If NI was demonstrated, then statistical superiority was tested under closed test procedure.
Results: Baseline characteristics (56 yrs, 6.5 yrs T2DM, HbA1c 8.1%, BMI 33 kg/m^2) were similar across groups. At 24 wks in the ITT population, reductions in HbA1c and FPG with Taspo10 and Taspo20 were superior to Exe (Table). More patients on Taspo10 or Taspo20 achieved target HbA1c ≤7% than Exe: 64.8% (95% confidence interval [CI], 59.8-69.6), 67.9% (95% CI, 63.0-72.5), and 51.5% (95% CI, 46.3-56.7); respectively. Dose-dependent weight loss was seen with Taspo and weight loss with Taspo20 was similar to Exe (Table). Gastrointestinal (GI) complaints were the most frequently reported adverse events across all arms. Although they occurred with a higher incidence in the Taspo groups, discontinuation rate due to GI events was similar in the 3 arms.
Conclusion: Once-weekly Taspo provided superior glycemic control to Exe, with similar body weight reduction at the higher dose and comparable tolerability. (NCT00717457)

LSMean ± SE	Taspo10 (n=384)	Taspo20 (n=392)	Exe (n=373)
Baseline HbA1c (%)	8.08±0.05	8.08±0.05	8.05±0.05
Change from baseline HbA1c (95% CI)	-1.24±0.09 (-1.41, -1.08)	-1.31±0.08 (-1.48, -1.15)	-0.98±0.08 (-1.14, -0.82)
Diff from Exe	-0.26±0.06**	-0.33±0.06**	_
24 Week HbA1c%	6.91±0.05	6.84±0.04	7.15±0.05
Baseline FPG (mmol/l)	9.91±0.13	9.83±0.13	9.87±0.13
Change from baseline FPG (95% CI)	-2.18±0.20 (-2.57, -1.79)	-2.48±0.20 (-2.87, -2.10)	-1.81±0.19 (-2.19, -1.43)
Diff from Exe	-0.37±0.14	-0.67±0.13	_
Baseline body weight (kg)	95.46±0.98	93.18±0.97	94.48±0.99
Change from baseline body weight (95% CI)	-1.60±0.37 (-2.33, -0.88)	-2.33±0.37 (-3.05, -1.60)	-2.27±0.36 (-2.98, -1.56)
Diff from Exe	0.67±0.25*	-0.05±0.25	_

*P<0.05; **P<0.0001

Supported by: Roche

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Retrospective cohort studies of the risk of acute pancreatitis: initiators of exenatide compared to other antidiabetic drugs in two commercial US health insurance claims databases

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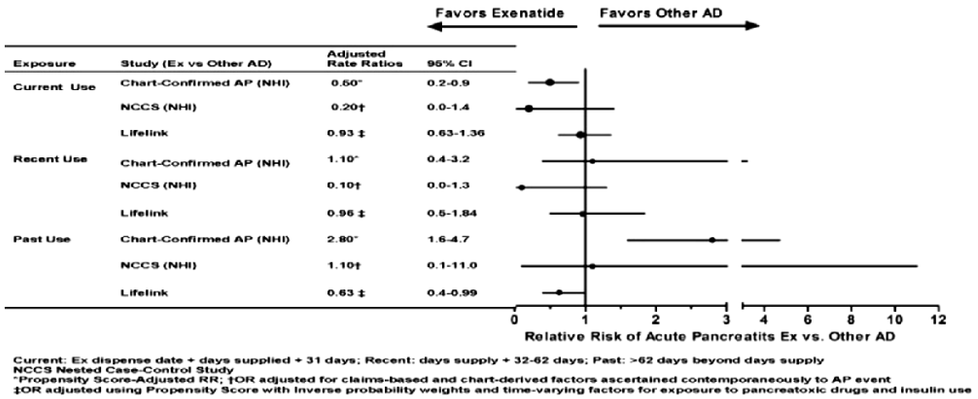
Background and aims: Cases of acute pancreatitis (AP) in patients treated with exenatide BID (Ex BID) have been reported.
Objective: To estimate the risk of AP in Ex BID initiators compared to initiators of other antidiabetic drugs (AD).
Materials and methods: Two retrospective studies were conducted to compare the rates of AP between patients without prior claims for pancreatic disease who initiated Ex BID or other AD (from 6/2005 to 12/2008) within 2 US commercial healthcare claims databases (Normative Health Information Database [NHI], Lifelink). The study protocols were similar to evaluate reproducibility of results. Both studies compared the risk of AP during periods of current, recent, or past exposure to Ex BID relative to other AD. AP cases were defined as patients with medical chart-confirmed acute pancreatitis (NHI) or claims for a hospitalisation associated with a primary diagnosis of AP (Lifelink). The NHI study used a multivariable Poisson regression model to estimate the AP propensity score adjusted rate ratios (RRs), and 95% confidence intervals (CIs) for current, recent, or past exposure. It included a blinded-medical record adjudication of claims-identified AP cases and a nested case-control study (NCCS) that involved conditional logistic regression modeling to obtain odds ratios (ORs) and 95% CIs adjusted for differences in the prevalence of risk factors between patients who persisted or discontinued the study drugs. The LifeLink study

analysis used discrete-time survival models to account for time-dependent exposure and inverse probability of treatment weighting to estimate ORs and 95% CIs for current, recent, or past Ex BID exposure accounting for measured risk factors.

Results: In both studies, Ex BID-treated patients were more likely to be female, obese, and had more evidence of diabetes complications than initiators of other AD. In the NHI database, there were 40 confirmed-AP cases in the Ex BID cohort (N=25,719), compared to 254 cases in the other AD cohort (N=234,536). In the LifeLink database, there were 46 AP cases in the Ex BID initiation cohort (N= 24,437) and 802 AP cases in the other AD cohort (N=457,797). In both analyses, current and recent Ex use periods were not associated with an increased risk of AP (Figure). For the past Ex BID use group in the NHI study, an increased risk of AP was observed, which was attenuated following NCCS adjustments for cholelithiasis, past AP, and obesity identified from the medical record review. However, in the LifeLink study, past Ex BID use was not associated with an increased risk.

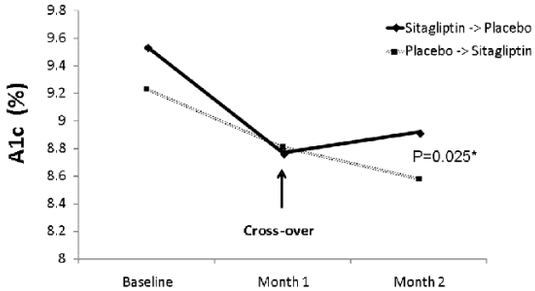
Conclusion: Two studies using distinct analytic methods and data sets found Ex use was not associated with an increased risk of AP.



Supported by: Amylin Pharmaceuticals, Inc. and Eli Lilly & Co.

involving larger sample size for a longer period is needed to determine efficacy and safety of sitagliptin in patients with type 1 diabetes.

Figure: A1c Values



*MANOVA controlling by period, treatment and insulin dose

Supported by: Merck & Co., Inc.

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Effect of sitagliptin on glucose control in patients with type 1 diabetes - a pilot study

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Background: Despite new therapies and technology, the average A1c in patients with type 1 diabetes (T1DM) remains well above ADA recommended targets. This investigator-initiated pilot study was designed to evaluate the effects of sitagliptin in poorly controlled (A1c 8.5-12%) patients with T1DM.

Methods: The study outcomes included area-under-the-curve for glucose excursions, A1c, and mean glucose values and other glycemic indices from CGM. Twenty patients were enrolled in this pilot randomized, double-blind, cross-over study to receive sitagliptin 100 mg daily or placebo for 1 month and then crossed over for 1 month. All patients used a blinded DexCom continuous glucose monitor (CGM) throughout the study period.

Results: There were no differences in baseline demographics between the two groups. Mean ± SD age and duration of diabetes were 32.5 ± 12.3 and 17.3 ± 7.5 years respectively. One patient was discontinued while on placebo for severe hypoglycemia. There was a significant reduction in insulin dose in subjects while on sitagliptin (p=0.02). Sitagliptin use reduced A1c values during both periods within groups. After controlling for period, treatment and insulin dose, there was a significant reduction in A1c in patients receiving sitagliptin (LSM = -0.27±0.11%; p=0.025; Figure). The CGM downloads showed a decrease in mean (± SE) blood glucose (-10.9 ± 3.8, p=0.012), J Index (-9.0 ± 3.1, p=0.010), High Blood Glucose Index (2.2 ± 0.70, p=0.007), M100 (-8.1 ± 2.7, p=0.009) and GRADE (-1.1 ± 0.4, p=0.023) when patients were receiving sitagliptin. Time spent in euglycemic range (80-140 mg/dl) was also significantly increased during sitagliptin use (0.46±0.20, p=0.046), while subjects trended to spending less time (hrs) in hyperglycemic range >240mg/dl (-0.55± 0.38, p=0.17).

Conclusions: We conclude that sitagliptin reduced total daily insulin dose, A1c and mean blood glucose values in patients with T1DM. Further research

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Comparing ITCA 650, continuous subcutaneous delivery of exenatide via DUROS® device, vs. twice daily exenatide injections in metformin-treated type 2 diabetes

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Background and aims: ITCA 650 provides for the continuous and consistent delivery of exenatide using the DUROS technology, a subcutaneous osmotic delivery system that can deliver exenatide for up to 12 months with a single administration. This provides the opportunity to escalate to optimal doses of exenatide with good tolerability and ensure 100% patient compliance.

Materials and methods: A phase 2 study to evaluate the efficacy, safety and tolerability of ITCA 650 treatment with an introductory treatment dose during the initial 12 weeks and subsequent dose escalation is being conducted in subjects with inadequately controlled, metformin-treated type 2 diabetes (T2DM). In this study, subjects (n=50 /group) were initially randomized to receive either 20 or 40 mcg/day of exenatide delivered by ITCA 650 for 12 weeks or twice daily exenatide injections at 5 mcg BID for 4 weeks followed by 10 mcg BID for 8 weeks. Subsequently, subjects were randomized to receive ITCA 650 at 20, 40, 60 or 80 mcg/day for an additional 12 weeks.

Results: Treatment with ITCA 650 was well tolerated with less reported nausea at 20 mcg/day relative to exenatide injections. HbA1c was significantly lower after 12 weeks in all treatment groups (p<0.001 at week 12 relative to baseline). Weight was also reduced in all treatment groups. Dose escalation to ITCA 650 at the higher doses of 60-80 mcg/day resulted in further reductions in HbA1c at week 16 with a change of -1.3% (n=36) relative to baseline and continued weight loss.

Conclusion: ITCA 650 represents a novel and attractive treatment to deliver exenatide in a well-tolerated fashion that ensures 100% compliance for the long-term treatment of T2DM, resulting in substantial changes in HbA1c and weight without the need for repeated self injections. Escalation to higher doses leads to further reductions in HbA1c and weight in the long-term treatment of T2DM.

HbA1c and Weight Loss at Week 12

Dose Group	n	Baseline HbA1c	HbA1c at Week 12	Change in HbA1c at Week 12	HbA1c ≤7% at Week 12	n	Change in weight at Week 12
ITCA 650 20 mcg/day	36	7.9% ±0.8%	7.1% ±0.8%	-0.8% ±0.9%	58%	33	-0.5% ±2.5%
ITCA 650 40 mcg/day	38	8.1% ±0.8%	7.1% ±0.8%	-1.0% ±0.7%	63%	38	-2.1% ±3.3%
Exenatide injections	38	8.0% ±0.9%	7.2% ±0.8%	-0.8% ±0.8%	53%	34	-1.9% ±2.3%

OP 14 Biomarkers and coronary heart disease risk

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Fluctuation of haemoglobin A_{1c} is associated with higher incidence of cardiovascular disease in patients with type 2 diabetes

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Background and aims: Recent studies demonstrated that variability of hemoglobin A1C (HbA1C) may be associated with the risk of diabetic micro- and macrovascular complications in patients with type 1 diabetes; however, whether the similar association may exist in patients with type 2 diabetes is unclear. We, therefore, conducted this cohort study to highlight the relationship between the fluctuation of HbA1C and incident cardiovascular disease (CVD) in patients with type 2 diabetes.
Materials and methods: We studied 689 Japanese type 2 diabetic patients with an estimated glomerular filtration rate (eGFR) ≥ 15 mL/min/1.73 m^2, including 295 women and 394 men (mean [± SD] age: 65 ± 11 years). Patients were observed at least 12 months. Variability of HbA1C was defined as the intrapersonal SD of serially measured HbA1C during the whole follow-up period. Patients were divided into quartiles of SD HbA1C. The primary endpoint was defined as incident CVD including cerebral infarction and hemorrhage, myocardial infarction, and angina pectoris requiring coronary revascularization. Cox proportional hazard model was used to calculate hazard ratio and 95% confidence interval (95% CI). In the multivariate Cox regression analysis, the following variables were incorporated and selected by the stepwise procedure; age, sex, duration of diabetes, presence of proliferative diabetic retinopathy, smoking status, use of renin-angiotensin system inhibitors, antiplatelet agents and statins, hemoglobin, uric acid, eGFR, urinary albumin-to-creatinine ratio (ACR) at baseline, the mean of body mass index, systolic and diastolic blood pressures, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol levels during the follow-up period, and the mean, SD and number of measured HbA1C.
Results: During a median follow-up period of 3.3 years (range: 1.0 - 6.3 years, 2,279 patient-years), 26 ± 14 measurements of HbA1C were obtained per patient, and incident CVD episodes were observed in 61 patients (26.8 episodes per 1,000 patient-years). Patients with higher quartiles of SD HbA1C had a higher incidence of CVD; five-year cumulative incidence of CVD in patients with the first, second, third, and fourth quartile in order of increasing were 4.9, 8.7, 17.1, and 26.2%, respectively ($p < 0.001$ by the log-rank test). In the multivariate Cox analysis, the fourth quartile of SD HbA1C was associated with significantly higher incidence of CVD (hazard ratio 3.38, 95% CI 1.07 - 10.63) *versus* the first quartile, independently of the mean of HbA1C and traditional cardiovascular risk factors. Other significant covariates remained in the Cox model were age (hazard ratio 1.03, 95% CI 1.00 - 1.07), logarithmically transformed urinary ACR (hazard ratio 2.24, 95% CI 1.58 - 3.17, $p < 0.001$), and history of CVD (hazard ratio 2.34, 95% CI 1.32 - 4.16, $p = 0.004$).
Conclusion: Visit-to-visit variability of HbA1C may be a potent predictor of incident CVD, independent of mean HbA1C in Japanese patients with type 2 diabetes.

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Significant association of TCF7L2 variant rs7903146 with angiographically characterised coronary artery disease in women

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Background and aims: Type 2 diabetes mellitus (T2DM) confers a particularly high risk of coronary artery disease (CAD) in women. Variations in the transcription factor 7-like 2 (TCF7L2) gene, particularly rs7903146, increase T2DM risk; their association with CAD is uncertain, no data are available for women. We therefore aimed at investigating potential links between TCF7L2 variant rs7903146 and CAD in women.

Materials and methods: We therefore investigated the association between rs7903146 and angiographically determined CAD in a cohort of 554 female Caucasian patients undergoing coronary angiography for the evaluation of established or suspected CAD. At angiography, significant CAD was diagnosed in the presence of significant coronary stenoses with lumen narrowing of ≥50%. The severity of CAD was calculated as the sum of all stenosis percentages of a given patient divided by the number of coronary stenoses in this patient and the extent as the number of significant coronary stenoses. The association between rs7903146 and CAD was evaluated in an additive genetic model.
Results: Variant rs7903146 was significantly associated with angiographically characterized CAD (additive odds ratio (OR)=1.37 [1.07-1.76]; p=0.011). Adjustment for age, smoking, BMI, total- and HDL-cholesterol did not significantly change this finding (OR)=1.37 [1.06-1.78]; p=0.016). Also, after further adjustment for T2DM, the association between rs7903146 and CAD remained significant (OR=1.31 [1.00-1.70]; p=0.047). Further, the extent of CAD significantly increased from subjects who were homozygous for the C allele over heterozygous subjects to those who carried the TT genotype (0.79±1.45, vs. 0.95±1.40 and 0.98±1.50, respectively; p=0.022). Similarly, a significant association between SNP rs7903146 and the severity of coronary lesions was observed (severity scores of 26.1±36.7, 35.7±39.9, and 36.7±39.4 for the CC, CT, and TT genotype, respectively; p=0.004).
Conclusion: We conclude that TCF7L2 variant rs7903146 is significantly associated with angiographically diagnosed CAD in women.

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Trajectories of adiponectin before coronary heart disease (CHD): the Whitehall II prospective cohort study

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Background and aims: Adiponectin, an adipocyte-specific cytokine, is associated with reduced risk of type 2 diabetes, and it has anti-inflammatory, anti-thrombotic and anti-atherogenic properties. Although coronary heart disease (CHD) is a major complication of type 2 diabetes, high adiponectin levels have not been consistently linked to reduced CHD risk. One hypothesis is that the association between adiponectin and CHD varies as a function of the disease process over time. Thus, we took advantage of data from repeated measurements of adiponectin (i) to examine how serum adiponectin concentrations change during years preceding the manifestation of CHD and (ii) to compare these trajectories with those from individuals who did not develop CHD.
Materials and methods: This is a case-cohort analysis within the Whitehall II cohort with a total of 2778 participants without CHD at baseline, 26.9% women, mean age at baseline 49.3 (SD 5.9) years and average BMI 25.2 (SD 3.5) kg/m2. There were 284 incident cases of CHD (myocardial infarction, angina) during follow-up and 2494 non-cases who remained CHD-free. Serum adiponectin was measured at up to three time points for each participant (phase 3, the baseline: 1991-94; phase 5: 1997-99; phase 7: 2003-04). Year 0 of the observation period was the year of CHD diagnosis for cases and a randomly selected time point during follow-up for non-cases to approximate the follow-up time distribution of cases. Adiponectin levels were traced backwards up to participants' first adiponectin measurement. Multilevel models adjusted for age, sex and ethnicity were fitted to assess changes in adiponectin during the preceding 13 years based on 398 and 3871 serum samples in cases and non-cases, respectively.
Results: Serum adiponectin levels were 6% lower in cases [mean (95% CI), 8457 (8021; 8916) ng/ml] than in non-cases [8989 (8837; 9151) ng/ml] at study baseline. This difference remained constant during the follow-up in a model adjusted for age, sex and ethnicity (p=0.03) which means that it was independent of time until CHD diagnosis. The addition of nonlinear time terms for CHD cases did not improve the fit of the multilevel models, nor did these terms reach statistical significance. After adjustment for BMI or waist circumference as time-varying covariates, the slope of the linear upward adiponectin trajectories remained similar both among cases and non-cases, however the difference between the two groups was no longer statistically significant.
Conclusion: Adiponectin is mildly, but significantly lower in individuals who develop CHD throughout the 13 years preceding disease onset compared to those who remained CHD-free. BMI or waist circumference accounted for a substantial part of this difference, suggesting that adiponectin does not predict CHD independently of obesity. We found no support for the hypothesis that the association between adiponectin and CHD varies as a function of the disease process.
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Vitamin D levels and asymptomatic coronary artery disease in type 2 diabetic patients with elevated urinary albumin excretion rate

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Background and aims: Coronary artery disease (CAD) is the major cause of morbidity and mortality in type 2 diabetic patients. Severe vitamin D deficiency has been shown to predict cardiovascular mortality in type 2 diabetic patients. We investigated the association between severe vitamin D deficiency and asymptomatic CAD in type 2 diabetic patients with elevated urinary albumin excretion rate (UAER) > 30mg/24h. Furthermore, we evaluated the association between severe vitamin D deficiency and coronary calcium score (CCS).
Materials and methods: A cross sectional study including 200 type 2 diabetic patients without clinical signs of CAD. Plasma 25-hydroxy-vitamin D levels were determined by high performance liquid chromatography/tandem mass spectrometry. Severe vitamin D deficiency was defined as plasma 25-hydroxy-vitamin D < 12.5 nmol/l. Patients with plasma NT-proBNP > 45.2 ng/L and/or CCS > 400 were arbitrarily stratified as high risk patients for CAD (n = 129). High risk patients were examined by myocardial perfusion imaging (MPI; n = 109), and/or CT-angiography (CTA; n = 20), and/or coronary angiography (CAG; n = 86).
Results: Patients received mulitifactorial treatment, yielding mean (SD) HbA_{1c} 7.9 (1.3) %, plasma total cholesterol 3.9 (0.9) mmol/l and arterial blood pressure 130 (17)/75 (11) mmHg.
Median (range) vitamin D level was 36.9 (3.8-118.6) nmol/l. Vitamin D was not associated with sex, blood pressure, HbA_{1c} and UAER but a weak positive association was found with age (R = 0.159, p = 0.025). The prevalence of severe vitamin D deficiency was 9.5% (19/200). In 70 (35%) patients significant CAD was demonstrated by MPI and/or CAG. In a logistic regression model, severe vitamin D deficiency was associated with asymptomatic CAD, unadjusted odds ratio (OR [95% CI] 2.24 [0.87-5.81]; p = 0.097). After adjusting for additional risk factors (sex, age, plasma total cholesterol, plasma creatinine, distal systolic blood pressure at toe level, vibratory perception threshold, heart rate variability and plasma NT-proBNP), the OR was 5.00 [1.28-19.49]; p = 0.020. The prevalence of CCS > 400 was 34% (68/200). Severe vitamin D deficiency was significantly associated with CCS > 400 (unadjusted OR 4.32 [1.54-12.10]; p = 0.005). The association persisted after adjusting for all additional risk factors, (OR 3.98 [1.20-13.113]; p = 0.023). Moderate vitamin D deficiency, plasma 25-hydroxy-vitamin D: 12.5-25 nmol/l, and vitamin D insufficiency plasma 25-hydroxy-vitamin D: 25-50 nmol/l, were not significantly associated with increased presence of CAD or CCS > 400.
Conclusion: In high risk type 2 diabetes patients with elevated urinary albumin excretion rate, low levels of vitamin D are strongly and independently associated with asymptomatic coronary artery disease.
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Prevalence and risk factors accounting for true silent myocardial ischaemia (clandestine ischaemia) in type 2 diabetic patients: a pilot case-control study

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Background and aims: Given the elevated risk of cardiovascular events and the higher prevalence of silent coronary artery disease (CAD) in diabetic versus non-diabetic patients, the need to screen asymptomatic diabetic patients for CAD assumes increasing importance. The term of silent ischemia includes an entity named true silent myocardial ischemia or clandestine myocardial ischemia, which is characterized by myocardial perfusion defects in the absence of both angina and ST-segment depression > 1 mm during the exercise test. To the best of our knowledge there have been no studies addressed to determining its prevalence and the risk factors associated with its development. The aims of the study were to assess prospectively the prevalence and clinical predictors of true silent myocardial ischemia or clandestine ischemia in asymptomatic type 2 diabetic patients.
Material and methods: Stress myocardial perfusion gated-SPECT (Single Photon Emission Computed Tomography) was carried out in 41 type 2 diabetic patients without history of cardiovascular disease and 41 nondiabetic patients matched by age and gender. Left ventricular end-diastolic and end-systolic volumes, and ejection fraction were also measured in gated-SPECT. Exclusion criteria were: 1) history of cardiovascular disease (CVD); 2) electrocardiographic evidence of Q-wave myocardial infarction, ischemic ST depression, T-wave changes, or complete left bundle branch block; 3) flat or downsloping ST segment depression > 1 mm at 80 ms after the J-point during an exercise test on a bicycle ergometer.
Results: Apart from diabetes there were no significant differences between the two groups regarding either the classic CVD risk factors (age, gender, smoking habit, dyslipemia, hypertension, and family history of CAD) or left ventricular function. Clandestine ischemia was detected in 21.9% of type 2 diabetic patients but only in 2.4% of controls (p<0.01). The presence of myocardial perfusion defects did not correlate with the number of risk factors but was independently associated with male gender and the presence of diabetic retinopathy (DR). The probability of having myocardial perfusion defects in an asymptomatic diabetic patient with DR was 11.7 [IC95%: 3.7-37].
Conclusion: We conclude that clandestine ischemia is frequent in asymptomatic type 2 diabetic patients. In addition, DR is a high risk condition for true silent myocardial ischemia in asymptomatic type 2 diabetic patients, and point to these patients as a target to be screened for cardiovascular disease. Further prospective studies evaluating the prognostic value of these findings and their impact in economical terms are warranted.
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Type 2 diabetes is not a coronary heart disease risk equivalent: results from an 8-year prospective cohort study on angiographically characterised coronary patients

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Background and aims: Current guidelines consider diabetes as a coronary artery disease (CAD) risk equivalent, but cardiovascular risk in patients with diabetes may vary substantially depending on the presence of subclinical CAD at baseline. In the present study we therefore aimed at investigating the contribution of baseline coronary atherosclerosis to the risk of future vascular events in patients with diabetes.
Materials and methods: Vascular events were recorded over 8 years in 750 consecutive patients undergoing coronary angiography for the evaluation of established or suspected stable CAD.
Results: From our patients, 244 had neither type 2 diabetes (T2DM) nor significant CAD (i.e. coronary stenoses ≥50%) at the baseline angiography, 50 had T2DM but not significant CAD, 342 did not have T2DM but had significant CAD, and 114 had both T2DM and significant CAD. Non-diabetic subjects without significant CAD had an event rate of 20.5%. The event rate was similar in T2DM patients without significant CAD (22.0%; p = 0.811), but higher in non-diabetic patients with significant CAD (39.5%, p <0.001). Patients with T2DM plus significant CAD had the highest event rate (53.5%; p <0.001). Importantly, T2DM patients without significant CAD had a significantly lower event rate than non-diabetic patients with significant CAD (p = 0.017).
Conclusion: T2DM *per se* is not a CAD risk equivalent. Moderate risk diabetic patients without significant CAD and very high-risk diabetic patients with significant CAD add up to a grand total of high risk diabetic patients: this is why diabetes appears as a CAD risk equivalent in many epidemiological studies.

OP 15 Manipulating the gut to treat metabolism

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Long-term prevention of mortality in morbid obesity through bariatric surgery. A systematic review and meta-analysis of trials performed with gastric banding and gastric bypass

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Background: Bariatric surgery has been reported to reduce long-term mortality in comparison with non-operated subjects, but there are no studies comparing gastric banding and gastric by-pass.
Methods: We performed a systematic review and meta-analysis of clinical trials published as full papers dealing with all-cause mortality, cardiovascular mortality, and global mortality (sum of all-cause and cardiovascular mortality). Pooled-random effects of estimates of the risk of mortality in subjects undergoing surgery, compared with controls, were calculated using the Der Simonian and Laird models.
Results: Of 44,022 participants from eight trials, death occurred in 3,317 subjects (400 in surgery, 2,917 in controls); when the kind of death was specified, 321 cardiovascular deaths (118 in surgery, 203 in controls), and 523 all-cause deaths (218 in surgery, 305 in controls) occurred. Compared with controls, surgery was associated with a reduced risk of global mortality (OR = 0.27, 95% C.I. 0.16-0.45), of cardiovascular mortality (OR = 0.43, C.I. 0.24-0.77), and of all-cause mortality (OR = 0.60, C.I. 0.37-0.97). Data of all-cause mortality were not heterogeneous; heterogeneity of data of cardiovascular mortality and of global mortality disappeared when studies were grouped according to size (larger vs smaller studies). At meta-regression analysis, decrease of global mortality (Log OR) was significantly associated with increasing BMI ($p = 0.036$). The effect of gastric banding and gastric by-pass (3,797 vs 10,255 interventions) was not significantly different for the any kind of mortality.
Conclusion: This meta-analysis indicates that: 1) bariatric surgery reduces all-cause, cardiovascular, and global mortality; 2) there are no significant differences between gastric banding and gastric by-pass; 3) the effect is more evident for greater BMI.

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Weight loss modulates DNA methylation of peroxisome proliferator-activated receptor gamma coactivator-1 and pyruvate dehydrogenase kinase, isozyme 4 promoters

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Background and aims: Obesity is associated with reduced insulin sensitivity and extensive changes in skeletal muscle. When diet and drugs no longer work, many morbidly obese individuals opt to undergo bariatric surgery as a means to reduce body mass. DNA methylation is believed to be modulated by the environment and can act as a reversible switch of gene expression that can lock genes in an active or repressed state. We determined whether DNA methylation of select proteins is altered in obesity and after weight loss.
Materials and methods: DNA methylation level of the Peroxisome Proliferator-Activated Receptor γ Coactivator-1 α (PGC-1α) and Pyruvate Dehydrogenase Kinase, isozyme 4 (PDK4) promoters was investigated in vastus lateralis skeletal muscle from eight non-diabetic obese people (41.8 ± 3.7 years) before (BMI 42.1 ± 1.5 kg/m^2) and six months after gastric bypass surgery (BMI 31.2 ± 1.6 kg/m^2).
Results: Plasma concentrations of glucose and insulin were reduced 6 months after weight loss surgery (5.6 ± 0.3 vs 4.8 ± 0.2 mmol/L and 99.9 ± 19.8 vs 56.6 ± 8.4 pmol/L, glucose and insulin, respectively). Levels of inflammatory cytokines such as C-reaction protein (CRP) and monocyte chemoattractant protein-1 (MCP-1) were markedly decreased after weight loss surgery. Weight loss induced hypomethylation of the PGC-1α promoter and hypermethylation of the PDK4 promoter as examined by bisulfite DNA methylation analysis. These changes in DNA methylation levels were inversely correlated with mRNA expression of PGC-1α and PDK4. To determine whether systemic factors associated with insulin resistance and obesity influence promoter methylation, we incubated primary human skeletal muscle cultures for 48 hours with tumor necrosis factor-α (TNF- α). In vitro exposure of muscle cells triggered hypermethylation of the PGC-1α promoter and hypomethylation of the PDK4 promoter.
Conclusion: The dynamic regulation of PGC-1α and PDK4 promoter methylation with obesity and after weight loss surgery appears to contribute to the regulation of mRNA expression and metabolism. Our results further suggest that environmental factors acutely alter the methylation status of promoters regulating glucose and lipid metabolism in skeletal muscle. DNA methylation in somatic cells appears to be a more dynamic process than previously thought.

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Assessment of insulin secretion and insulin sensitivity with the hyperglycaemic clamp in type 2 diabetic patients with BMI below 35 Kg/m^2 submitted to ileal interposition associated to sleeve gastrectomy

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Background and aims: Type 2 diabetes is a progressive disease. Beta cell failure is related to the reduction in insulin secretion and associated with some complications of the disease. Laparoscopic ileal interposition associated with sleeve gastrectomy (II-SG) had demonstrated a positive effect in insulin resistance. We used the hyperglycemic clamp, the gold standard method for measurement of insulin secretion to assess the impact of this operation on beta cell function.
Materials and methods: A 3-hour hyperglycemic clamp was performed in 35 patients preoperatively and in 33 patients postoperatively. To calculate the first phase insulin secretion (1st phase), an intravenous glucose load was made at the beginning. In the 170 minutes remaining, glucose was kept 125 mg / dl above basal glucose, in order to calculate the second phase insulin secretion (2nd phase). Mean age was 55 years (range 34-69 years). Mean diabetes duration was 12.3 years (5-24 years). Mean follow-up was 20 months (range 8-38 months).
Results: Postoperatively, BMI decreased from 29.5 +-5.2 Kg/m^2 to 22.1 +- 2.4 Kg/m^2. Mean plasma glucose was 259 mg/dl in preoperative and 239 mg/dl in postoperative period. Insulin sensitivity increased from 69.4 mmol.min^{-1}.nmol^{-1} to 95.5 mmol.min^{-1}.nmol^{-1}. In the 1st phase, the acute C-peptide response was 2219 +- 2268 pmol.l^{-1} .10min and increased to 2617 +- 2164 pmol.l^{-1} .10min. The relation of incremental C-peptide area under the curve and incremental glucose area under the curve, increased from 30 +- 24 pmol.mmol-1 to 46 +- 30 pmol.mmol-1. The evaluation of the 2nd phase by the insulin incremental area and insulin sensitivity was 688 x 10^4 pmol.mmol.nmol^{-1} and increased to 811 x 10^4 pmol.mmol.nmol^{-1} postoperatively.
Conclusion: In non morbidly obese type 2 diabetic patients ileal interposition associated with sleeve gastrectomy showed an important improvement in insulin secretion after a mean follow-up of 20 months as demonstrated by the hyperglycemic clamp.

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The role of apolipoprotein A5 in non-alcoholic fatty liver disease

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Background and aims: The term non-alcoholic fatty liver disease (NAFLD) comprises a wide spectrum of clinical entities ranging from simple steatosis, steatohepatitis to fibrosis and cirrhosis. NAFLD is strongly associated with obesity and is found in up to 91% of severe obese patients undergoing bariatric surgery. Apolipoprotein (apo) A5 is a recently discovered protein synthesized by the liver that plays a major role in triglyceride metabolism. In mice, overexpression of apoA5 leads to dramatically decreased plasma triglyceride levels. Mechanistically, extracellular effects by activating lipoprotein lipase and intracellular effects have been proposed. In this study we aimed to investigate a possible role of apoA5 in NAFLD.
Materials and methods: Hepatic apoA5 mRNA expression was determined in 15 severely obese subjects with histologically proven NAFLD before and after pronounced weight loss due to bariatric surgery. Hepatic apoA5 mRNA expression was estimated by fluorescence-based real time polymerase chain reaction. Effects of apoA5 on hepatic triglyceride accumulation were investigated in apoA5 deficient HepG2 cells due to transfection with apoA5 siRNA.
Results: Mean weight loss of 18 kg was accompanied by improved insulin sensitivity as estimated by the HOMA index and improvements in hepatic steatosis. In parallel, hepatic apoA5 mRNA expression was decreased by 41 % ($p=0.017$). Transfection of apoA5 siRNA in HepG2 led to a mean reduction

of 83 % in apoA5 mRNA expression (p=0.001), as determined by fluorescence-based real time PCR. Suppression of apoA5 in HepG2 cells resulted in a reduction of intracellular triglyceride content by 31 % (p<0.001), when compared to controls transfected with nonsilencing siRNA.
Conclusion: In summary, we found that pronounced weight loss in obese subjects with NAFLD results in a significant improvement of hepatic steatosis which is associated with a decline of hepatic apoA5 mRNA levels. Our in vitro data suggest that weight loss induced reduction in hepatic apoA5 mRNA expression is not an epiphenomenon but has a causal role in improvement of NAFLD in morbid obesity.
Supported by: ÖDG

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Can diabetes and obesity be treated through the rectum?
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Background and aims: The enteroendocrine L cells produce glucagon gene products including GLP-1 and oxyntomodulin which are satiety factors and the former is an insulin secretagogue. The L-cells also produce PYY, the major molecular form of which is also a satiety factor. The number of L-cells and hormonal contents increase distally through the gut with highest concentrations in the rectum. We have previously shown that intracolonic infusion of bile salts in humans causes secretion of L-cell hormones, triggered via TGR5 membrane receptors. The present study was designed to investigate the dose-responsive effects of intrarectal taurocholic acid (TA) on circulating concentrations of GLP-1, PYY, insulin, glucose and on food intake of a subsequent meal.
Materials and methods: Ten obese type 2 diabetic subjects were each studied on five separate occasions after an overnight fast and administration of 100 mg oral sitagliptin10 hours before the study. They then received an intrarectal infusion of either one of four amounts of TA (0.66, 2, 6.66, or 20 mmoles) or vehicle placebo in a random blinded fashion. Bile salts were administered in 20 ml of a 1% carboxymethyl cellulose emulsion over 1 min. Hormone and glucose measurement was made in plasma samples were collected for one hour following the infusion. 75 mins after the infusion, the subjects were presented with an unlimited amount of a previously selected favorite meal and invited to eat until satisfied.
Results: TA caused a dose-responsive increase of GLP-1, PYY and insulin, with peak concentrations ~ 7-fold, 4-fold and 3-fold increased, respectively with 20 moles TA (all P<.0001). There was a corresponding decrease in circulating glucose concentrations (P< 0.0001), with a decrease in glucose of ~2.8 mmol/L with 20 moles TA. The TA infusions were also associated with a decrease in caloric consumption of the subsequent meal (P<0.0001), with a maximal inhibition of 45±6 % with 20 mmoles TA.
Conclusion: Intrarectal TA increased L cell secretion which markedly decreased food intake and, since the subjects had fasting hyperglycemia, this resulted in an incretin effect. These findings probably explain the glucose-lowering effects of bile salt sequestrants. Intrarectal TGR5 agonists are likely to be valuable in the treatment of type 2 diabetes and obesity.
Supported by: Satiogen Pharmaceuticals

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Metabolic effects of transplanting gut microbiota from lean donors to subjects with metabolic syndrome
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Background: Recent data in animal models revealed that obesity is associated with substantial changes in composition and metabolic function of gut microbiota. Moreover, colonization of germ-free mice with faeces harvested from obese mice resulted in a significantly greater increase in total body fat than colonization with a 'lean microbiota'. However, data on the role of gut-microbiota in human obesity are scarce. Thus, our aim was to examine the effect of faecal infusions derived from lean healthy donors on gut microbiota composition, glucose and lipids in metabolic syndrome.
Methods: This study was a double-blind, randomised controlled trial. A total of 18 male subjects with newly diagnosed metabolic syndrome (BMI≥30 kg/m2, FPG>5.6mmol/L, TG>1,6 mmol/L with no medication use) underwent jejunum biopsies and subsequent polyethylene-glycol bowel lavage through duodenal tube followed by random assignment to either allogenic (from lean male donors with BMI<23 kg/m2, n=9) or autologous faecal transplantation (reinfusion of own collected faeces, n=9). We studied changes in sigmoidal microbiota composition and fasting lipidprofiles at 0.5, 2, 6 and 12 weeks after faecal transplantation. Weight, jejunal gut microbiota (epithelial biopsy) and glucose metabolism (peripheral and hepatic insulin sensitivity as assessed by hyperinsulinemic euglycemic clamp with stable isotopes) were studied before and 6 weeks after transplantation.
Results: Lean subjects were characterized by different sigmoidal gutmicrobiota compared to obese subjects (by HITChip phylogenetic microarray analysis). Fasting levels of TG-rich lipoproteins (TG/ApoB ratio) were significantly reduced following donor faeces (1.43 ± 0.21 to 1.11 ± 0.18, p<0.01) with no effect after autologous faeces infusion. REE and basal endogenous glucose production (EGP) did not change in both groups after faecal infusion. Although weight remained stable, an improvement in both peripheral (Rd) and hepatic insulin sensitivity (suppression of EGP) was found 6 weeks after allogenic faeces (median Rd: from 26.2 to 45.3 µmol/kg.min, p=0.02 and EGP suppression: from 51.5 to 61.6 %, p=0.08) while no significant changes were observed in the autologous treatment group (Rd: from 21.0 to 19.5 µmol/kg.min and EGP suppression:from 53.8 to 52.4 %, ns). Changes in jejunal microbiota are currently analyzed.
Conclusion: Lean donor faecal infusion improves hepatic and peripheral insulin resistance as well as fasting lipid levels in obese individuals with the metabolic syndrome underscoring the potential role of gut microbiota in the disturbances of glucose and lipid metabolism in obesity. Our data could provide pathophysiological insight in the metabolic deviations in obese subjects and a rationale for therapeutic intervention.
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OP 16 Mechanisms of insulin secretion

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The role of wnt antagonists in impaired beta cell exocytosis

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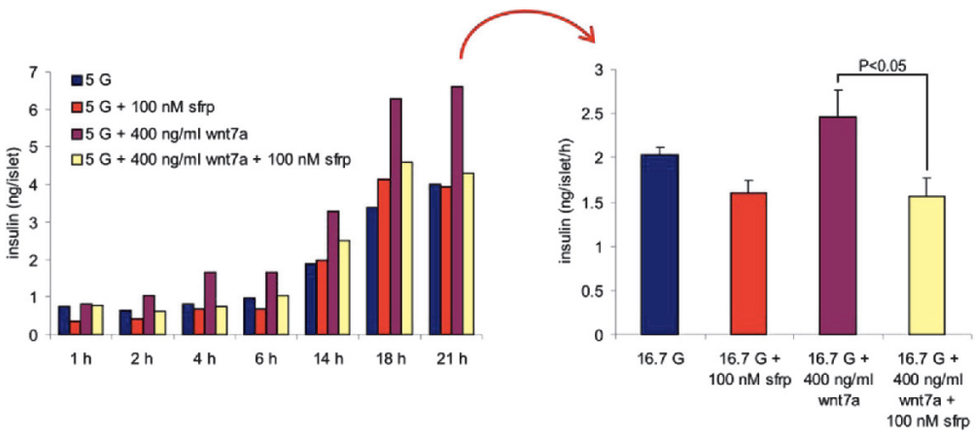
Background and aims: We have recently found that genetically encoded overexpression of ADRA2A (the alpha2A-adrenergic receptor) contributes to impaired insulin secretion in type 2 diabetes (T2D). The aim here is to get a more overall view of the adrenergic signaling system and linked molecular pathways in defective insulin release. Network methods have proven valuable for studying how cellular pathways cause disease and have been successfully applied to e.g. obesity. However, in the case of T2D little is known about the networks that drive the pathogenesis.
Materials and methods: We have used linear algebraic methods to analyze the topological overlap in microarray data from human islets and performed subsequent cluster analysis. Highly connected hub genes play an important role in networks, and hub gene validation has been conducted by insulin secretion assays and patch-clamp recordings of exocytosis.
Results: We have characterized the gene expression networks in human islets and identified a subnetwork with 120 genes connected to ADRA2A. The expression of this subnetwork was associated with reduced insulin secretion ($p=0.008$), elevated HbA1c ($p=0.0002$) and T2D ($p=0.00006$) in the islet donors. Of the top 100 genes for which the expression was correlated with T2D in the microarray, as many as 53 belonged to the identified ADRA2A-related network (enrichment p value is 8e-189). One of the most highly connected hub genes of the ADRA2A subnetwork is a secreted frizzled-related protein (sfrp), which is an extracellular antagonist of the wnt system. Interestingly, TCF7L2, the strongest T2D gene known, is regulated by the wnt system. The role of sfrp in T2D has not been studied previously. Our data show that activation of the wnt system by wnt7a stimulated insulin secretion both during a 21 hour incubation at low glucose (5 mM) and when glucose thereafter was elevated to 16.7 mM (see Figure). Interestingly, sfrp has previously been shown to co-precipitate with wnt7a in neuron studies, and the addition of sfrp completely antagonized the stimulatory effect of wnt7a in islets ($p<0.05$). These findings were paralleled on the single-cell level. Beta-cell exocytosis increased from 12.5±2.2 fF/pF to 20.6±3.3 fF/pF ($p=0.045$) by pre-treatment with 400 ng/ml wnt7a. The stimulatory effect of wnt7a was abolished by the addition of 100 nM sfrp (10.2±4.1 fF/pF). Sfrp also had an inhibitory effect on its own and reduced exocytosis to 5.9±1.4 fF/pF ($p=0.013$ vs. control, and $p=0.0002$ vs. wnt7a).
Conclusion: We have characterized the gene expression networks in human islets and identified an ADRA2A-related subnetwork that is associated with T2D. One of the subnetwork hub genes, sfrp, reduced insulin secretion and beta-cell exocytosis when applied alone or in combination with wnt activators. This validates the importance of the identified subnetwork in T2D and illuminates an extracellular player that could affect insulin secretion through the TCF7L2 pathway.



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Resveratrol potentiates glucose-stimulated insulin secretion in INS-1E cells and human islets through SIRT1 dependent mechanism

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Background and aims: Sirtuins are energy sensors which mediate effects of calorie restriction-induced lifespan extension. SIRT1, one of the mammalian Sirtuin homolog, is a transcription factor that deacetylates specific substrates. Previous studies have shown that SIRT1 is implicated in glucose metabolism and beta-cell function. Here, we investigated the role of SIRT1 through the activating action of resveratrol on insulin secretion. Resveratrol is a polyphenol found in red grapes and wine that has been demonstrated to increase lifespan and to protect mice against high-fat diet induced obesity and insulin resistance.
Materials and methods: Chronic (24h) treatment with 25 μM resveratrol was tested on insulinoma INS-1E cells and effects on insulin secretion, gene expression (qRT-PCR), and metabolic parameters were measured (ATP production, glucose oxidation and utilization, oxygen consumption). We then tested resveratrol treatment in cells over-expressing SIRT1 or expressing inactive mutant SIRT1 after adenoviral transduction, as well as pharmacological SIRT1 inhibitor (1 μM EX-527). Chronic effects of resveratrol on insulin secretion and gene expression were also studied in human islets.
Results: Chronic treatment of INS-1E cells with 25 μM resveratrol resulted in marked potentiation of insulin secretion at 15 mM glucose (+114%, $p<0.01$). This effect was associated with enhanced metabolism since glucose utilization was increased by +32% ($p<0.01$), resulting in elevated glucose oxidation, ATP generation, and mitochondrial oxygen consumption. Such changes correlated with up-regulation of key genes for beta-cell function and differentiation, i.e. glucose transporter GLUT2 (+59%), glucokinase (+89%), Pdx-1 (+70%), and TFAM (+232%). In human islets, chronic resveratrol treatment also significantly increased glucose-stimulated insulin secretion and induced expression of the same set of genes. Over-expression of SIRT1 in INS-1E cells potentiated resveratrol effects on insulin secretion. Conversely, inhibition of SIRT1 achieved either by expression of a mutant that lacks deacetylase activity or alternatively by using the EX-527 inhibitor, both abolished resveratrol effects on glucose-stimulated insulin secretion. Chronic treatment of INS-1E cells with EX-527 also prevented up-regulation of GLUT2, glucokinase, Pdx-1, and TFAM, normally induced by resveratrol.
Conclusion: Treatment of beta-cells with resveratrol markedly enhanced glucose-induced insulin secretion in INS-1E cells and human islets, even after removal of the compound from the medium. Data show that resveratrol effects were mediated by SIRT1 activation, defining a new role for SIRT1 in the regulation of insulin secretion.

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Melatonin effects on insulin secretion and clock genes expression

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Background and aims: Melatonin is rhythmically released by the pineal gland and thus represents an important regulator of seasonal and circadian rhythms. Both melatonin receptors MTNR1A and MTNR1B are expressed in INS-1 cells and in rat and human islets (primarily in alpha cells). Genome-wide association studies have identified a SNP, rs10830963, in *MTNR1B* that is associated with elevated fasting plasma glucose levels, impaired insulin secretion and T2D. Previously, peripheral circadian rhythms in heart, liver and pancreas have been shown and that these rhythms are independent from the central clock located in the suprachiasmatic nucleus (SCN). Given the association of *MTNR1B* with T2D and insulin secretion, we aimed to resolve whether the effects of melatonin on β-cells could be mediated by entrainment of circadian rhythm.
Materials and methods: Expression of mRNA for Clock genes in human islets and clonal β-cells (INS-1 832/13 cells) was determined by microarray (Affymetrix) or RT-PCR, respectively. To determine the effects of melatonin on insulin secretion, we incubated human islets and clonal β-cells at 2.8 and 16.7 mM glucose with or without 0.1 μM melatonin for 1 h. Released insulin was determined by radioimmunoassay.
Results: Microarray analysis of human islets showed that Clock, Per1-3, and Cry 1-2 were expressed. Moreover, while MTNR1A expression correlated positively with Per1 (rho=0.037; p=0.04), MTNR1B expression correlated negatively with Cry1 (rho=-0.41; p=0.02) in the human islets. RT-PCR confirmed mRNA expression of the melatonin receptors MTNR1A and MTNR1B in INS-1 832/13 cells. In addition, mRNA expression of the clock genes CLOCK and PER1 were detected in INS-1 832/13 cells. Insulin from 832/13 cells increased 6.92-fold in response to16.7 mM glucose (69.65 ± 9.11 insulin ng/mg protein/h) compared to secretion at 2.8 mM glucose (10.07 ± 2.35 insulin ng/mg protein/h). The stimulation of the INS-1 832/13 cells with glucose + melatonin resulted in decreased GSIS (4.57 fold; p=0.02) at 16.7 mM glucose (36.54 ± 8.72 insulin ng/mg protein/h). Insulin from human islets, from 4 different donors, decreased 2-fold (p=0.06) in response to 16.7 mM glucose + melatonin (0.79 ± 0.17 insulin ng /islet /h) compared to secretion at 16.7 mM glucose (1.34 ± 0.18 insulin ng /islet /h).
Conclusion: Our data demonstrate the existence of components of the molecular clock in human islets and clonal β-cells. In addition, melatonin exerted an inhibitory effect on insulin secretion provoked by high glucose. Given that islets and β-cells express CLOCK genes and are sensitive to melatonin, there is a possibility that melatonin may entrain circadian rhythm. This agrees with previous suggestions that insulin displays a diurnal pattern.

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Quantitative analysis of t-SNARE and Ca2+-channel clusters near secretory granules

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Background and aims: For insulin to be secreted from pancreatic -cells, secretory granules must translocate to and then dock at the plasma membrane. Secretagogues can then trigger exocytosis of these granules, resulting in release of insulin. Only a limited number of granules can dock at any time, suggesting the existence of specialized docking sites where the exocytosis machinery is assembled. This is supported by the finding that the t-SNARE syntaxin 1a (syx), an integral plasma membrane protein which is required for docking and exocytosis, clusters in raft-like nanodomains near docked granules. The function of these clusters is unknown, but it seems likely that they contain other t-SNARE and regulatory proteins, which together act as acceptor complex for the docking granule. Moreover, there is indirect evidence suggesting that clusters of L-type Ca2+-channels (CaV1.2) are required near the granule for rapid exocytosis; ablation of the gene encoding Cav1.2 abolishes 1st phase insulin secretion and results in systemic glucose intolerance. The channel associates in biochemical assays with t-SNAREs. Here we have quantified the dynamic interaction of syx and CaV1.2 clusters with secretory granules during docking and exocytosis.
Materials and methods: Dual-color TIRF microscopy and novel quantitative image analysis was used to study the association of GFP-labeled syx and Cav1.2 with granules at the plasma membrane of live Ins1- and PC12 cells. Granules were labeled with NPY-cherry as spatial reference. Single molecule imaging and PALM super-resolution microscopy were done on the same instrument to measure protein diffusion and cluster size.
Results: Syx and CaV1.2 formed small clusters (<100 nm) in the plasma membrane that contained about 70 molecules of syntaxin and associated with a subset of the docked granules (30-50%). Granules near such a cluster were more likely to undergo exocytosis during stimulation. Clusters were stable on a minute scale and moved no faster than docked granules. In contrast, the number of syx molecules fluctuated on a second scale and individual molecules of both proteins diffused rapidly in the plasma membrane. When observed at the single molecule level, individual copies of syx and Cav1.2 were captured beneath docked granules, for a short time (<1s). Syx was recruited to the granule site during docking, and lost during undocking and exocytosis. A syx mutant lacking the N-terminus formed clusters that were excluded from granules, while a mutation in the SNARE domain had little effect.
Conclusion: In summary, the protein composition of individual granule-associated nanodomains is remarkably dynamic and correlates with the granules' ability to exocytose. This organization is established during or just after granule docking, which suggests that granules approaching the plasma membrane might induce the formation of their own docking site. Dynamic association of exocytosis proteins with individual granules occurs on a timescale consistent with rapid cellular signaling, and may be important for the short-term regulation of insulin secretion.
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CART is upregulated in islets of type 2 diabetic patients, stimulates insulin secretion, inhibits glucagon secretion and protects against beta cell death

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Background and aims: Cocaine- and amphetamine-regulated transcript (CART) is a regulatory peptide expressed in the islets of many laboratory animal species. CART KO mice exhibit impaired glucose tolerance due to islet dysfunction, and CART has been shown to regulate insulin secretion from rat islets and clonal beta cells. Furthermore, CART is upregulated in rodent models of type 2 diabetes (T2D). We examined CART expression in human islets from T2D patients and control subjects. Furthermore, we studied the effect of CART on beta cell survival, as well as the effect of CART on insulin and glucagon secretion in vivo and in vitro in mice. We have previously shown that CART exerts its effect on insulin secretion in part via increased cAMP and PKA-dependent pathways. In this study we investigated potential effects of CART on Ca2+ signaling.
Materials and methods: CART expression was examined using immunocytochemistry, in situ hybridization. IVGTT with i.v. administration of CART was performed in anesthetized C57Bl6 mice. Insulin and glucagon secretion after 1h static incubations of isolated mouse islets was analyzed with RIA or ELISA. Ca2+ fluorescence and glucotoxicity was assessed in INS-1 (832/13) cells.
Results: CART mRNA and protein expression was evident in human alpha and beta cells as well as in intra-pancreatic nerve fibers innervating the islets. T2D islets displayed 3-fold higher CART mRNA levels than controls. In addition, the number of CART-expressing alpha and beta cells was 2.5-fold higher in type 2 diabetic patients, as compared to control subjects. Intravenous CART administration improved glucose-elimination and increased glucose stimulated insulin secretion (GSIS) after an IVGTT in mice. CART increased insulin secretion from isolated mouse islets stimulated with glucose, GLP-1, and palmitate. On the other hand, CART had no effect on insulin secretion in the presence of KCl, alpha-KIC or carbachol. CART increased cytoplasmic Ca2+ concentration in INS-1 (832/13) cells in a dose-dependent fashion at both high and low glucose concentrations. The effect was still evident when Ca2+ was absent in the assay buffer, suggesting that Ca2+ is mobilized from internal stores. The effect of CART was sustained after prior exposure to pertussis toxin, suggesting that CART does not act via Gi/o coupled receptors. In addition, CART reduced secretion of glucagon from isolated mouse islets at both low and high glucose concentrations. Furthermore, addition of ex-

ogenous CART resulted in a 50% decrease in glucose-induced cell death in INS-1 (832/13) cells.
Conclusion: We conclude that CART 1) is expressed in human islet cells and nerve fibers. 2) is upregulated in the islets of type 2 diabetic subjects. 3) improves glucose elimination and stimulates insulin secretion in vivo and in vitro. 4) inhibits glucagon secretion. 5) exerts a cytoprotective effect on beta cells. Together these data suggest that CART is upregulated in human T2D islets to protect the islets against glucotoxicity and to lower plasma glucose.
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Dysfunctions of neuronal NO synthase in pancreatic beta cells from human islets issued from obese subjects
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Background and aims: We have previously shown that rat pancreatic β-cells express an isoform of neuronal NO synthase (nNOS), that controls insulin secretion through two catalytic activities: NO production and cytochrome c reduction. We also found functional and molecular abnormalities of nNOS, involved in pancreatic β-cell hyperactivity in an animal model of prediabetic state, the Zucker fa/fa rat. In the present study, we investigate whether, as observed in rats, nNOS dysfunctions could also occur in islets from obese subjects.
Materials and methods: Human islets from normal-weight or obese subjects were obtained from brain dead donors in the frame of a transplantation protocol of Gragil network. nNOS expression was studied by RT-PCR and Western Blot, its subcellular localization by confocal microscopy. Insulin secretion in response to glucose (2.8, 8.3, and 16.7 mM) was evaluated on pools of 10 islets during 90 minutes in the presence or not of a pharmacological inhibitor of nNOS, Nω-nitro-L-arginine methyl ester (L-NAME).
Results: We found that nNOS is expressed in human β-cells and, as previously observed in rats, co-locates with insulin secretory granules. In islets from obese subjects, nNOS is overexpressed and co-locates more strongly with insulin granules as compared to islets from normal-weight subjects. Moreover, islets from obese subjects were found more sensitive to glucose, as previously observed in fa/fat rats: at 2.8 mM glucose, insulin secretion reached 19.4 ± 2.05 (n = 2), versus 10.09 ± 0.8 ng/ml/10 islets/90 min ($p < 0.001$) for islets from lean subjects (n = 3), 31.25 ± 2.8 versus 15.3 ± 1.3 ng/ml/10 islets/90 min ($p < 0.001$) at 8.3 mM glucose and 31.2 ± 3.2 versus 21.35 ± 1.9 ng/ml/10 islets/90 min ($p < 0.01$) at 16.7 mM glucose. Finally, in islets issued from lean subjects, pharmacological blockade of nNOS with 10 mM L-NAME stimulated insulin secretion from 15.3 ± 1.3 to 22.4 ± 2.4 ng/ml/10 islets/90 min ($p < 0.01$) at 8.3 mM glucose and from 21.35 ± 1.9 to 27.7 ± 2.8 ng/ml/10 islets/90 min ($p < 0.05$) at 16.7 mM glucose, confirming that nNOS exerts an inhibitory tone on insulin secretion. In contrast, islets from obese subjects were found insensitive to L-NAME, whatever the glucose concentration studied, suggesting a constitutive defect of nNOS.
Conclusion: Islets from obese subjects display molecular and functional abnormalities of nNOS that could be involved in early dysfunctions observed in these islets, as previously observed in an animal model of prediabetic state.

OP 17 Role of mitochondria in muscle insulin action

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High oxidative capacity protects against lipid-induced insulin resistance
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Introduction: Fat accumulation in skeletal muscle combined with low mitochondrial oxidative capacity, is associated with muscular insulin resistance. Endurance trained athletes, characterized by high oxidative capacity, have elevated amounts of intramyocellular lipid, yet are highly insulin sensitive. This suggests that a high oxidative capacity may prevent lipid-induced insulin resistance. We examined whether athletes are protected against lipid-induced insulin resistance. In addition, we examined whether ex vivo mitochondrial function deteriorates upon elevation of circulating fatty acids in healthy young men.
Methods: Nine endurance-trained athletes and 10 control subjects with a sedentary lifestyle were included and matched for age (23.4 ± 0.9 and 21.9 ± 0.9 y). Subjects underwent a 6h hyperinsulinemic-euglycemic clamp with simultaneous infusion of a triglyceride emulsion (intralipid) or glycerol. The rate of insulin-stimulated glucose disposal (Rd), oxidative (CHOox) and non-oxidative glucose (NOGD) disposal were computed as the difference between insulin stimulated conditions minus non-insulin stimulated conditions. Muscle biopsies from the m. vastus lateralis were taken before and after the clamp to measure ex vivo mitochondrial function in the addition of several substrate combinations. Mitochondrial function was determined as ADP-stimulated respiration, uncoupled respiration and maximal oxidative capacity, all expressed as pmol/(s*mg)/ mtDNA copy number.
Results: Endurance trained subjects had higher VO2max values compared to control subjects (61.5 ± 1.2 vs 43.0 ± 1.1 ml kg-1 min-1, $p<0.01$). Interestingly, although mitochondrial content was higher in trained subjects, ex vivo mitochondrial function normalized to mtDNA copy number was similar between trained and control subjects. Rd was 63% lower after intralipid compared to glycerol in control subjects (from 35.7 ± 2.7 to 12.0 ± 3.1 μmol kg-1 min-1, $p<0.05$). In contrast, Rd reduced only 29% upon lipid in trained subject (from 45.9 ± 3.6 to 31.6 ± 3.9 μmol kg-1 min-1, $p<0.05$), which was different from untrained subjects ($p<0.05$). The stimulation of CHO-ox by insulin as seen in the glycerol condition was completely blunted after intralipid in both groups. Interestingly, in trained subjects insulin-stimulated NOGD was unaffected upon intralipid, but reduced by 52% in control subjects (from 29.1 ± 2.7 to 14.0 ± 3.4 μmol kg-1 min-1, $p<0.05$). Finally, in both groups respiration values upon intralipid were not different from after glycerol and were unaffected by training status.
Discussion: We show that lipid-induced insulin resistance is blunted in athletes. Interestingly, lipid infusion reduced the insulin-stimulated increase in carbohydrate oxidation rates to a similar extend in trained and untrained subjects, but the non-oxidative glucose disposal was completely unaffected in athletes. These results suggest that a high oxidative capacity prevents lipid-induced reduction of non-oxidative glucose storage and thereby blunts lipid-induced insulin resistance. Higher oxidative capacity in athletes was due to higher mitochondrial density and not intrinsic mitochondrial function, suggesting that the latter may be of less importance in lipid-induced insulin resistance. Finally, mitochondrial function does not deteriorate upon the infusion of lipids.
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Adiponectin and AdipoR1 regulate PGC-1alpha and mitochondria by Ca2+ signalling and AMPK/SIRT1 like exercise
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Background and aims: Adiponectin is an anti-diabetic adipokine. Its receptors AdipoRs possess a seven-transmembrane topology with the amino terminus located intracellularly, which is opposite to G-protein coupled receptors. Intracellular signal transduction mechanisms of AdipoRs have yet to be well clarified. Insulin resistance has been reported to be associated with impaired skeletal muscle oxidation capacity and mitochondrial dysfunction. However,

the exact cause of mitochondrial dysfunction has yet to be ascertained. We attempted to clarify whether decreased adiponectin/AdipoR1 signalling could be associated with mitochondrial dysfunction, and attempted to elucidate the signalling mechanisms by which adiponectin/AdipoR1 would exert their biological effects.
Materials and methods: We analyzed muscle-specific AdipoR1-knockout mice.
Results: Adiponectin induces extracellular Ca2+ influx via AdipoR1, which was necessary for subsequent activation of Ca2+ /calmodulin-dependent protein kinase kinase β (CaMKKβ) and AMPK/SIRT1, increased expression and decreased acetylation of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), and increased mitochondria in myocytes. Next we showed that muscle-specific disruption of AdipoR1 resulted in decreased elevation of the intracellular Ca2+ concentration and decreased activation of CaMKK/AMPK/SIRT1 by adiponectin as well as decreased expression and increased acetylation of PGC-1 alpha, decreased mitochondrial content and enzymes such as cytochrome c (CytC), decreased oxidative type I myofibers, decreased oxidative stress-detoxifying enzymes such as catalase and manganese superoxide dismutase (SOD2), and decreased molecules involved in fatty-acid oxidation such as medium-chain acyl-CoA dehydrogenase (MCAD), thereby leading to increased oxidative stress and increased tissue triglyceride content in skeletal muscle. Furthermore, muscle-specific AdipoR1 knockout mice exhibited increased phosphorylation of p70 S6 kinase and JNK and also increased serine phosphorylation of IRS-1 as well as decreased glucose transporter (GLUT)4 expression and decreased Akt activation by insulin, which were associated with decreased rates of glucose disposal (Rd). Importantly, these alterations could result in insulin resistance and decreased exercise endurance. Moreover, AdipoR1 and AdipoR2 double knockout mice as well as obese diabetic db/db mice exhibited almost all the same phenotypes in skeletal muscle observed in muscle-specific AdipoR1 knockout mice. Interestingly, AMPK activator AICAR could only partially rescue the phenotypes of muscle-specific AdipoR1 knockout mice such as insulin resistance and decreased mitochondrial content and function, whereas exercise almost completely rescue their phenotypes.
Conclusion: AdipoR1 appears to 1) regulate mitochondrial function and oxidative stress in muscle as well as insulin sensitivity and exercise endurance, and 2) be required for adiponectin-induced PGC-1α expression and activation via extracellular Ca2+ influx and AMPK/SIRT1, respectively, and subsequent mitochondrial bioenergetics stimulated with adiponectin in muscle cells. Decreased levels of adiponectin/AdipoR1 in obesity may play causal roles in mitochondrial dysfunction and insulin resistance seen in diabetes. Agonism of AdipoR1 as well as strategies to increase AdipoR1 in muscle could be exercise-mimetics.

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Acute overexpression of PGC-1β in rat skeletal muscle increases mitochondrial substrate oxidation and ameliorates lipid-induced insulin resistance

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Background and aims: Lipid accumulation in skeletal muscle is strongly associated with the development of insulin resistance. In particular, reactive lipid species, such as diacylgycerols (DAG), ceramides, and long-chain acyl-CoAs (LCACoA) are thought to antagonize numerous intracellular pathways, which ultimately leads to reductions in insulin sensitivity. Recent data has also implicated oxidative stress as a key determinant in this process. Upregulation of mitochondrial capacity has been suggested as a potential approach to combat lipid overload and insulin resistance in skeletal muscle, however there is relatively little direct data to support this concept. In the current study we aimed to acutely overexpress PGC-1β in hindlimb skeletal muscle from chow and high-fat fed rats, and investigate the effect on mitochondrial function and insulin sensitivity.
Materials and methods: Rats were fed either a low-fat or high-fat diet for 4wk, and *in vivo* electroporation was used to overexpress PGC-1β in the tibialis cranialis and extensor digitorum longus muscles. Downstream effects of PGC-1β on markers of mitochondrial oxidative capacity and muscle lipid levels were characterized, and insulin action was examined *ex vivo* using intact muscle strips and *in vivo* via a hyperinsulinemic-euglycemic clamp.
Results: PGC-1β expression was increased >100% over basal levels. This upregulated the expression of many metabolic proteins, including those involved in mitochondrial function (ETC complexes I and II), lipid metabolism (CD36, CPT-1) and antioxidant defences (SOD-1, SOD-2). Additionally, the activity of oxidative enzymes and substrate oxidation (pyruvate and palmitate) was increased in muscles overexpressing PGC-1β. LCACoA was increased 2.3 fold in control muscles of high-fat fed rats, but remained similar to chow levels in muscles overexpressing PGC-1β ($p<0.05$, n=7). Under hyperinsulinemic-euglycemic clamp conditions, insulin-stimulated glucose uptake was decreased in tibialis cranialis (-20%, $p<0.05$) and extensor digitorum longus (EDL; -28%, $p<0.01$) muscles of high-fat fed rats, and was partially restored towards control levels with PGC-1β overexpression ($p<0.01$). Furthermore in isolated EDL strips, PGC-1β overexpression ameliorated the reduced insulin-stimulated glucose uptake observed with high-fat feeding ($p<0.01$). Finally, in PGC-1β overexpressing muscles we observed a significant decrease in two measures of oxidative stress; lipid peroxidation (-14% in chow fed, -19% in high-fat fed, $p<0.05$) and protein carbonylation (-15% in chow fed $p<0.05$).
Conclusion: These studies demonstrate that physiological overexpression of the transcriptional coactivator PGC-1β in rat skeletal muscle *in vivo* activates a coordinated subset of genes involved in mitochondrial function and substrate oxidation. This prevents diet-induced LCACoA accumulation, ameliorates oxidative damage, and significantly improves insulin action in the skeletal muscle.
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Knockdown of hypoxia-inducible factor-1 alpha ameliorates insulin-stimulated glucose uptake in the skeletal muscle cells

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Background and aim: Impaired insulin sensitivity in peripheral tissues comprises a major cause of hyperglycemia in type 2 diabetes mellitus. Skeletal muscle is the largest organ responsible for insulin-dependent glucose disposal thus plays important part in the regulation of blood glucose homeostasis. Molecular mechanism determining glucose uptake by skeletal muscle in response to insulin, however, remains largely unknown. The hypoxia-inducible factor-1alpha (HIF-1alpha) is a transcription factor operating in the cellular homeostatic regulation under hypoxic conditions. Recent studies have uncovered pleiotropic actions of HIF-1alpha in response to various cellular stimuli including insulin under normoxic condition. Physiological relevance of HIF-1alpha in regulation of insulin action in the target organs, however, has not been well documented. In the present study, we examined the role of HIF-1α in regulation of glucose uptake by the skeletal muscle cells.
Materials and Methods: C2C12 myoblast in which HIF-1alpha is knocked-down (C2C12-deltaHIF) was generated by a stable transfection with HIF-1alpha shRNA expression plasmids. C2C12-deltaHIF myoblasts were differentiated into myotubes then the glucose uptake rate of the cells treated with insulin was determined by monitoring the influx of [^{3}H]-2-deoxy glucose (2-DG).
Results: Upon the treatment with 100nM insulin for 30 minutes, wild type C2C12 myotube increased uptake of 2-DG by 2-fold compared to the basal level. Knockdown of HIF-1alpha in the myotubes (C2C12-deltaHIF) resulted in abrogation of insulin-dependent glucose uptake. Accordingly, in C2C12-deltaHIF myotubes GLUT4 translocation to plasma membrane by treatment with insulin was severely inhibited. The Akt substrate of 160 kDa (AS160), a Rab-GTPase activating protein is known to be responsible for GLUT4 vesicle transport. Immunoprecipitation assays demonstrated insulin-dependent phosphorylation of AS160 in wild type C2C12 myoutubes. Contrary, in C2C12-deltaHIF myotube phosphorylation of AS160 by insulin was significantly reduced. On the other hand, adiponectin, an adipose-derived cytokines, induced GLUT4 translocation and glucose uptake in an additive manner to insulin to demonstrate its insulin-sensitizing action in wild type C2C12 myoutubes. Of interest, both insulin and adiponectin enhanced HIF-1alpha expression in the wild type myotubes. On the other hand, in C2C12-deltaHIF myotubes adiponectin did not demonstrate any additive enhancing effect on glucose uptake by insulin. Finally, we determined glucose uptake by the skeletal muscles of heterozygote of HIF-1alpha gene knock-out mice (HIF-1alpha$^{+/-}$). Extensor digitorum longus (EDL) muscles were isolated from the wild type mice and HIF-1alpha$^{+/-}$ mice to carryout *ex vivo* culture. Insulin stimulated 2-DG uptake was increased up to 3-fold above basal levels in wild type mice. In contrast, HIF-1alpha $^{+/-}$ mice demonstrated significantly reduced uptake of 2-DG either in basal or in insulin-stimulated condition.

Conclusion: HIF-1alpha is a critical determinant for insulin sensitivity and glucose disposal in the skeletal muscle thus as a possible target to alleviate insulin resistance in type 2 diabetes.

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Deletion of the Rab-GAP protein TBC1D1 protects from lipid-induced insulin resistance in skeletal muscle

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Background and aims: The Rab-GTPase-activating (GAP) protein TBC1D1 is highly expressed in skeletal muscle and was recently linked to severe human obesity. We previously described its role as susceptibility gene for high-fat diet-induced obesity and diabetes in mice. TBC1D1-deficient recombinant congenic mice displayed reduced adiposity and a lowered respiratory quotient (RQ), indicating enhanced whole body lipid use. Consequently, intact isolated skeletal muscles from these animals showed increased fatty acid (FA) oxidation with concomitant decrease of insulin-stimulated glucose uptake, indicating that TBC1D1 regulates substrate preference in skeletal muscle. An impaired adaptation of skeletal muscle fuel preference to the availability of glucose and FA as energy substrates has been associated with the development of insulin resistance and diabetes. We therefore sought to investigate the role of TBC1D1 in glucose and lipid metabolism and the mediation of lipid-induced insulin resistance in skeletal muscle.
Materials and methods: C2C12 myotubes were electroporated with siRNA oligonucleotides to achieve *Tbc1d1* knockdown and subsequently exposed to different concentrations (0 - 750 μM) of palmitic acid for 16 h. In addition, intact skeletal muscles (EDL and soleus) from TBC1D1-deficient mice and wildtype controls were isolated and preincubated with increasing concentrations of palmitate. Insulin-stimulated 2-deoxyglucose (DOG) uptake and palmitate oxidation was determined using radioactive tracer techniques. Expression analysis was performed by quantitative real-time PCR and Western Blot.
Results: In cultured C2C12 myotubes, siRNA-mediated knockdown of *Tbc1d1* increased palmitate oxidation by approx. 30%. Likewise, isolated muscles from TBC1D1-deficient mice showed increased palmitate oxidation compared to muscles from wildtype littermates. In both cultured cells and isolated muscles, the enhanced FA combustion was accompanied by increased levels of mRNA for genes involved in lipid metabolism including *Acadl*, *Cd36*, *Ppargc1*, and *Fabp3*. Importantly, additional knockdown of *Cd36* in *Tbc1d1*-depleted C2C12 myotubes completely abrogated the increase in palmitate uptake. Exposure of C2C12 myotubes and isolated skeletal muscles to palmitic acid led to a substantial and dose-dependent reduction in insulin-stimulated AKT phosphorylation, and expression of *Ppargc1* mRNA, respectively. As a result, in the presence of 1 mM palmitate, the insulin-stimulated DOG uptake in isolated skeletal muscles was reduced by >50%. In contrast, depletion of TBC1D1 almost completely prevented the detrimental effect of palmitate on insulin-stimulated AKT phosphorylation, *Ppargc1* expression and insulin-stimulated DOG uptake, and thus restored insulin sensitivity in muscle cells.
Conclusion: Our data demonstrate that TBC1D1 deficiency protects skeletal muscle from lipid-induced insulin resistance, presumably by enhancing FA oxidation. In cultured myotubes, the fatty acid translocase FAT/CD36 is essential for TBC1D1-mediated FA uptake. Furthermore, we show that TBC1D1 exerts control on fuel selection and energy metabolism in skeletal muscle at least in part through the coordinated regulation of gene expression of key enzymes for lipid uptake and oxidation.
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Electrical pulse stimulation of human skeletal muscle cells mimics exercise and prevents insulin resistance induced by adipocyte-conditioned medium

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Background and aims: Skeletal muscle is one of the major insulin sensitive tissues and is responsible for about 80 % of the postprandial insulin-stimulated glucose uptake. Obesity is closely associated with muscle insulin resistance and a major risk factor for the pathogenesis of type II diabetes. Regular physical activity does not only prevent obesity, but also considerably improves insulin sensitivity and skeletal muscle metabolism. Exercise leads to activation of AMPK, enhances insulin signalling and improves glucose uptake. The aim of our project is to establish and characterise an in vitro model of human skeletal muscle contraction to study signalling pathways and mechanisms, which are involved in beneficial effects of muscle activity.
Materials and methods: Differentiated primary human skeletal muscle cells (hSKM cells) were stimulated with a C-pace pulse generator for up to 24 h (1 Hz, 11.5 V, 2 ms pulse duration), and immunofluorescence staining of sacromeric alpha-actinin was used to visualise the cytoskeleton. To induce insulin resistance, hSKM cells were incubated with adipocyte-derived conditioned media (CM) for 8 h. Afterwards, the cells were stimulated with 100 nM insulin for 10 min and lysed. Protein expression and phosphorylation of Akt and AMPK were analysed by SDS-PAGE and western blotting.
Results: After 2-3 h few myotubes started to contract and after 24 h most of the myotubes showed noticable contractile activity. While the protein expression level of sarcomeric alpha-actinin did not change, immunofluorescence staining showed de novo formation of sarcomeres with typical striated pattern. During contraction, AMPK phosphorylation increased over time and was significantly elevated after 8 h of contraction (5fold, n = 6). In addition, electric pulse stimulation of hSKM cells for 24 h led to increased secretion of IL-6 which was 2fold elevated compared to unstimulated controls (114 ± 40 pg/ml to 221 ± 68 pg/ml, n = 6). The incubation of hSKM cells with CM significantly reduced the insulin-stimulated phosphorylation of Akt (Ser473) by 35 % compared to non-treated controls. However, when the cells were pulse-stimulated during the incubation with CM the effect of CM on insulin-stimulated Akt phosphorylation was abrogated.
Conclusion: Our data show that the effects of electric pulse stimulation on hSKM cells were similar to the effect of exercise on skeletal muscle in terms of enhanced AMPK activation and IL-6 secretion. Furthermore, we observed de novo formation of functional active sarcomeric structures. The CM-induced impairment of insulin signalling could be prevented by contractile activity of hSKM cells. This result provides a direct evidence for the beneficial effect of muscle contraction activity in order to improve insulin sensitivity in conditions of insulin resistance. In summary, our model provides a unique tool to investigate mechanisms and underlying signalling pathways which mediate the beneficial effects of muscle contraction, and will help to further clarify the potential of exercise to combat insulin resistance.

OP 18 Diabetic nephropathy - experimental

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Effect of CB2 receptor activation in experimental diabetic nephropathy

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Objective: Diabetic nephropathy (DN) is characterised by increased glomerular permeability to proteins and excessive extracellular matrix accumulation in the mesangium, resulting eventually in glomerulosclerosis and progressive renal impairment. Endogenous cannabinoids (EC), anandamide and 2-arachidonoylglycerol, bind to two endocannabinoid receptors, named CB1 and CB2. We have recently reported that the CB1 receptor is overexpressed by podocytes in experimental diabetes and that CB1 blockade prevents podocyte protein downregulation and reduces albuminuria, indicating a deleterious effect of EC signalling through the CB1 receptor. Coexpression of both the CB1 and the CB2 receptors has been reported in several cell types. In addition, recent studies have shown that activation of the CB2 receptor has protective effects in experimental models of atherosclerosis, liver fibrosis, and cardiac ischemia/reperfusion injury through prevention of inflammatory and profibrotic processes. Therefore, our aim was to study if the CB2 receptor is expressed within the glomeruli and the effect of CB2 activation in experimental diabetic nephropathy.
Methods: Male C57Bl6 mice were made diabetic by intraperitoneal (IP) injection of streptozotocin at a dose of 55 mg/kg in citrate buffer delivered in 5 consecutive days. Control mice were injected with citrate buffer alone. After the onset of diabetes both control (ND n=13) and diabetic mice (DM n=21) were further randomised to receive treatment with either AM1241, a selective CB2-receptor agonist (3 mg/kg IP daily), or vehicle. 14 weeks after the induction of diabetes, mice were individually placed in metabolic cages for urine collections and blood samples taken for blood glucose and glycated haemoglobin measurements. Then, mice were sacrificed, kidneys removed, weighed, and analysed. Urinary albumin excretion was measured by enzyme-linked immunosorbent assay. CB2 receptor protein expression was studied by immunohistochemistry. Nephrin, synaptopodin, zonula occludens-1 (ZO-1) mRNA and protein expression were assessed by immunofluorescence and real-time PCR, respectively. Fibronectin, CTGF, and TGF-β1 mRNA levels were quantitated by real-time PCR on total renal cortex.
Results: The CB2 receptor was expressed within the glomeruli in a predominant podocyte distribution. Diabetes was associated with reduced body weight and elevations in both plasma glucose and glycated haemoglobin levels, but no differences were seen between treated and untreated mice. Albuminuria was significantly ($p<0.001$) increased in the diabetic animals [DM:296.86 (252.8-356.2) μg/18hrs, geometric mean (25^{th}-75^{th} percentile)] as compared to the controls [ND:78.87 (73.8-86.6)] and ameliorated by treatment with AM1241 [ND+AM1241:67.75 (59.9-77.3); DM+AM1241:183.59 (144.8-243.5); $p<0.01$ DM vs DM+AM1241]. In the diabetic mice the increase in albuminuria was paralleled a significant three-fold reduction in both nephrin and ZO-1 protein expression and this effect was completely prevented in mice treated with AM1241. Similarly, diabetes-induced downregulation of both nephrin and ZO-1 mRNA levels was abolished by AM1241. By contrast in diabetic mice AM1241 administration did not affect fibronectin, TGF-β1, and CTGF overexpression.
Conclusion: These findings demonstrate that the CB2 receptor is expressed within the glomeruli and that CB2 activation ameliorates the diabetic proteinuria, possibly via prevention of podocyte slit diaphragm protein loss.
Supported by: SID

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Poly(ADP-ribose) polymerase-1 (PARP-1) gene deficiency alleviates diabetic kidney disease

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Background and aims: Evidence for the important role of poly(ADP-ribose)polymerase-1 (PARP-1) in chronic diabetic complications is emerging. Several structurally unrelated PARP-1 inhibitors have been reported to prevent or alleviate diabetic nephropathy. This study evaluated further the role for PARP-1 in diabetic kidney disease using the PARP-1-deficient mouse.
Materials and methods: PARP-1-/- and the wild-type (129S1/SvImJ) mice were made diabetic with streptozotocin, 40 mg $kg^{-1}d^{-1}$, i.p., for, at least, 7 consecutive days, and were maintained for 12 weeks. PARP-1 and poly(ADP-ribosyl)ated protein levels were evaluated by Western blot analyses, and urinary albumin excretion and renal cortex nitrotyrosine and transforming growth factor-β_1 (TGF-β_1) concentrations by ELISA. Glomerular collagen deposition and mesangial expansion (PAS-positive staining) were evaluated by histochemistry, followed by quantitation with the Threshold Colour plug-in of ImageJ 1.43q and ImageJ 1.43q programs, respectively. Podocyte nuclei were detected with an anti-WT1 antibody, by immunohistochemistry. Podocyte numbers were counted per glomerular section, and 20-25 glomeruli were examined for each animal.
Results: Final blood glucose concentrations were ~3.7-fold higher in both diabetic groups than in controls. PARP-1 and poly(ADP-ribosyl)ated protein levels in the renal cortex were similar in non-diabetic and diabetic wild-type mice (100% and 107%) whereas all knockouts were PARP-1-negative. Poly(ADP-ribosyl)ated proteins, however, have been identified in all groups, and their levels were increased by 18% and 14% in diabetic wild-type and diabetic PARP-1-/- mice, compared with the corresponding controls. Diabetes was associated with ~21-fold and ~13-fold increases in albuminuria in wild-type and PARP-1-/- mice, respectively. PARP-1 gene deficiency prevented diabetes-induced kidney hypertrophy and alleviated mesangial expansion and collagen deposition. Renal podocyte loss, and nitrotyrosine and transforming growth factor-β_1 accumulations were slightly lower in diabetic PARP-1-/- mice, but the differences with the diabetic wild-type group did not achieve statistical significance.
Conclusion: PARP-/- gene deficiency alleviates although does not completely prevent diabetic kidney disease. The lack of a complete prevention could be related to the presence of other PARPs responsible for poly(ADP-ribosyl)ation in the renal cortex in PARP-1-deficient mouse and its increase in diabetes, and/or streptozotocin nephrotoxicity in mice.
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TIMP3 deficiency accelerates diabetic nephropathy

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Background and aims: Some experimental models of nephropathy have recently shown that activation of TACE/ADAM17 is a key step in the progression of glomerulopathy, even though the exact role of ADAM17 and its inhibitor TIMP3in a hyperglycemic environment is still unclear.
Materials and methods: To test this hypothesis we treated WT mice with streptozocin to induce hyperglycemia, and then measured TACE activity in kidney; we found a significant activation of the enzyme in hyperglycemic (STZ, streptozocin-treated) mice compared to untreated controls ($p<0.001$). Next, we analyzed STZ-WT and STZ-*Timp-3*$^{-/-}$ kidneys by IHC, real time PCR and WB.
Results: STZ-*Timp-3*$^{-/-}$ mice showed significantly increased mean glomerular area and fractional mesangial area ($p<0.05$ and $p<0.001$, respectively). STZ-*Timp-3*$^{-/-}$ mice showed also a thicker glomerular basement membrane, due to an increased amount of type IV collagen deposition ($p<0.01$), that is one of the early feature of diabetic nephropathy. Moreover, they also showed increased macrophage infiltration, measured by F4/80 staining ($p<0.01$), resulting in inflammation. Oxidative stress markers staining revealed that STZ-*Timp-3*$^{-/-}$ mice are characterized by an increased expression of NOX4, RAGE and N-Carboxymethyl-lysine, a major product of oxidative modification of glycated proteins ($p<0.01$ for all). To further analyze the role of TIMP3 in diabetic nephropathy at a molecular level we performed real time PCR on RNA samples derived from kidneys of STZ-*Timp-3*$^{-/-}$ and WT mice. We found that the expression of MCP-1, a marker of inflammation, was significantly increased in STZ-*Timp-3*$^{-/-}$ mice, while the expression of nephrin and WT1, two markers of podocyte functions, is reduced in STZ-*Timp-3*$^{-/-}$ mice compared to the WT littermates ($p<0.01$ for all). We also performed gene profiling experiments on kidneys of the same animals, and we could observe up-regulation of different classes of genes involved in inflammation (*Cxcl9*, *Ccr5*, *Mcp-1*, *Mcp-5*, *Aif-1, Cd36, Mgl-1, Mgl-2*), renal cells proliferation and fibrosis (*Pdgf-d*, *Tgfβ3*, *Fgf, Ghr*), lipid metabolism (*Fabp5, Fasn, Ldlr, Acaca, Acms3*) and transport (*Slc13a1, Slc7a13, Slc7a6, Slco4c1, Slc12a3, Glut8*) in STZ-*Timp-3*$^{-/-}$ mice compared to WT ones. We also found a significant reduction in the expression of genes connected to control of oxidative stress such

as *Foxo1 and Foxo3a* in the kidney of STZ-*Timp-3*$^{-/-}$ mice. Finally, we used a proteomic approach to identify proteins differentially expressed in kidneys of STZ- *Timp-3*$^{-/-}$ and WT mice. Proteomic analysis of total homogenates revealed the presence of the 78 kDa glucose-regulated protein (GRP78) and protein disulfide-isomerase A3 only in kidneys from STZ-treated Timp-3$^{-/-}$ mice, indicating an increased levels of endoplasmic reticulum stress in these mice compared to WT ones.
Conclusion: In conclusion, our preliminary results show that Timp3 plays a protective role in preventing inflammation and oxidative stress accumulation in kidney and, doing so, it can interfere with the development and progression of diabetic renal nephropathy.
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D-carnosine prevents diabetic nephropathy in db/db mice
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Background and aims: The advanced glycation end products (AGEs) are believed to participate in the pathogenesis of diabetic nephropathy by causing both direct modification of enzymatic activity, ligand binding, half-life and immunogenicity of proteins and receptor-mediated pro-inflammatory and pro-fibrotic effects. The endogenous dipeptide L-carnosine was shown to act as a quencher of reactive carbonyl AGE precursors. However, in humans, it is rapidly inactivated by carnosinase. The carnosinase-resistant compound D-carnosine (DC) have shown high activity, selectivity and plasma stability. Preliminary studies from our group have demonstrated that DC is effective in attenuating high fat diet-induced atherosclerosis in ApoE null mice. This study was aimed at evaluating the efficacy of DC in preventing diabetic nephropathy in *db/db* mice.
Materials and methods: Adults (aged 6 weeks) male *db/db* mice and the corresponding *db/m* controls were treated with a DC derivative (60 mg/kg body weight in the drinking water; Flamma S.p.A.) or vehicle for 14 weeks. At the end of this period, urines were collected for assessment of proteinuria, then mice were killed and both kidneys were sampled. Glomerular sclerosis index (GSI) was assessed semiquantitatively, then mean glomerular area (mGA) and fractional mesangial area (fMA) were measured by computer-assisted morphometry, with estimation of mean glomerular volume (mGV) from mGA and calculation of mean mesangial area (mMA) by the formula: (fMA x mGA)/100. Renal expression of inflammatory and disease progression markers as well as kidney content of HNE adducts and AGE receptors were assessed by immunohistochemistry and/or RT-PCR. Serum AGEs and isoprostane-8-epi-$PGF_{2\alpha}$ were measured by ELISA, pentosidine by HPLC and total carbonylated proteins (PCOs) by slot blot immunoassay.
Results: DC treatment induced a significant attenuation of renal disease in *db/db* mice, whereas it did not influence renal structure in *db/m* control mice. Proteinuria (-35%), GSI (-31%), mGA (3.22 ± 0.27 vs. 3.52 ± 0.21 $\mu m^2 x10^3$), mGV (137.5 ± 10.1 vs. 157.2 ± 13.7 $\mu m^3 x10^3$), mMA (428.0 ± 82.5 vs. 588.5 ± 48.7 μm^2) and fMA (13.2 ± 1.8 vs. 16.8 ± 1.7%) decreased significantly ($P<0.001$) in DC-treated *db/db* mice, as compared with untreated animals. Glomerular staining for HNE adducts (5.6 ± 2.7 vs. 13.5 ± 2.8 % of glomerular area) as well as for the extracellular matrix proteins fibronectin (11.7 ± 2.6 vs. 19.7 ± 1.8 % of glomerular area) and collagen IV (13.2 ± 3.4 vs. 24.6 ± 3.1 % of glomerular area) were also significantly reduced ($P<0.001$) in DC-treated vs. untreated *db/db* mice. The mRNA expression levels of F4/80, CXCR3, MCP-1, TNF-α, CHOP, and the AGE receptors RAGE, galectin-3 and CD36 were also significantly lower in *db/db* mice treated with DC. Finally, serum AGE, pentosidine, PCOs, and isoprostane-8-epi-$PGF_{2\alpha}$ levels were lower in DC-treated vs. untreated *db/db* mice.
Conclusion: These data show that DC is effective in reducing carbonyl reactive species and preventing renal injury in *db/db* mice, thus suggesting that carbonyl stress plays a major role in diabetic nephropathy and that DC derivatives might be useful for treatment of this complication.

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Glucagon like peptide-1 agonist, exendin-4, exerts anti-inflammatory effect on macrophage and glomerular endothelial cell through inhibition of NF-κB
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Background and aims: Glucagon like peptide-1 (GLP-1) is known to have various extrapancreatic effects in addition to enhancement of insulin secretion. We have recently shown the renoprotective effects of exendin-4 through anti-inflammatory effects.Exendin-4 suppressed macrophage infiltration, expression of ICAM-1 and type IV collagen and oxidative stress independent of blood glucose lowering actions in diabetic rats. In addition, we have demonstrated that GLP-1 receptor is expressed on glomerular endothelial cells and monocytes/macrophages. The aim of this study is to clarify the mechanism for the protective effects of exendin-4 against diabetic nephropathy.
Materials and methods: Five-week old male Sprague-Dawley rats were divided into four groups: non-diabetic: ND, non-diabetic rats treated with exendin-4: ND+EX, diabetic rats without treatment: DM, diabetic rats treated with exendin-4: DM+EX. Rats were administered intraperitoneally with exendin-4 (10μg/kg/day, ND+EX and DM+EX) or vehicle (ND and DM) every day for 8 weeks. To investigate the mechanisms of anti-inflammatory effects of exendin-4, we evaluated the nuclear factor-κB, p65DNA binding activity in the kidney, which is one of the most important transcription factors regulating both inflammation and oxidative stress. In addition to direct effects of exendin-4 on macrophages and endothelial cells, we examined pro-inflammatory cytokine expressions (TNF-α and IL-1β mRNA) in THP-1 cells by 15mM high glucose for 72 hours and ICAM-1 mRNA expression in human glomerular endothelial cells by 100pg/ml TNF-α for six hours. Furthermore, to confirm whether the effects of GLP-1 directly acted on GLP-1 receptor, we used the GLP-1 receptor antagonist.
Results: The activation of NF-κB p65DNA binding activity in the renal cortex was significantly enhanced in DM group compared with non-diabetic groups. Exendin-4 treatment significantly inhibited the NF-κB p65 DNA binding activity. Stimulation with high glucose enhanced gene expression of TNF-α and IL-1β inTHP-1 cells. Exendin-4 significantly attenuated TNF-α and IL-1β gene expression with dose-dependent manner. The effects of exendin-4 on TNF-α and IL-1β were blocked by GLP-1R antagonist. TNF-α enhanced ICAM-1 gene expression on human glomerular endothelial cells. Exendin-4 dose-dependently attenuated ICAM-1 gene expression. The effect of exendin-4 was blocked by GLP-1R antagonist.
Conclusion: The current results indicate that exendin-4 suppresses the activation of NF-κB in diabetic kidney. Exendin-4 directly acts on GLP-1 receptors and down-regulates the expression of cytokines and ICAM-1 on macrophage and glomerular endothelial cell. Exendin-4 may exert protective effects against diabetic nephropathy by inhibition of the interaction between macrophages and glomerular endothelial cells which promotes inflammatory process.
Supported by: Scientific Research from the Ministry of Education, Science, Culture, Sport

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Altered expression profiles of microRNA in the glomeruli of diabetic mice
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Background and aims: The postulated mechanism in the development of diabetic nephropathy involves several factors including protein kinase C activation, TGF-β, AGE, oxidative stress and microinflammation which were induced by long-term exposure to high glucose. However, the therapeutic strategy for diabetic nephropathy has not fully developed, thus, more detail molecular mechanism in the pathogenesis of diabetic nephropathy and new treatment are still desired. The microRNAs, short non-coding RNAs of 21 to 23 nucleotides, have recently been shown to play an important role on biological process, including development, differentiation and proliferation, in various types of cells. We examined microRNA expression profile in renal glomeruli of diabetic mice to clarify the pathogenesis and find a novel therapeutic target of diabetic nephropathy.

Materials and methods: Eight-week-old male C57BL/6J mice were used. Diabetes were induced by intraperitoneal injection with streptozotocin at 200 mg/kg in citrate buffer (DM, n=6). Non-diabetic control mice were injected with citrate buffer (ND, n=5). Blood pressure, blood glucose, HbA1c and urinary excretion of albumin were measured every month. Kidneys were harvested at 6 months after induction of diabetes. Renal glomeruli were isolated magnetically after intra-arterial injection of iron oxide. MicroRNAs were extracted by miRNeasy Mini Kit. Agilent microRNA array (567 mouse microRNAs) was used to analyze the microRNA expression profile.
Results: We identified the total 21 microRNAs that showed a specific expression pattern in DM mice compared with ND mice. Up-regulated microRNAs (1.5 > DM/ND) in DM renal glomeruli were 8 genes (mmu-miR-705, mmu-miR-714, mmu-miR-34a, mmu-miR-21, mmu-miR-221, mmu-miR-141, mmu-miR-689, mmu-miR-671-5p). Down-regulated microRNAs (0.75 < DM/ND) in DM renal glomeruli were 13 genes (mmu-miR-1, mmu-miR-219b-5p, mmu-miR-503, mmu-miR-574-5p, mmu-miR-133b, mmu-miR-197, mmu-miR-466i, mmu-miR-467d, mmu-miR-466f-3p, mmu-miR-574-3p, mmu-miR-335-5p, mmu-miR-322*, mmu-miR-1187).
Conclusion: We identified the microRNAs which showed a specific expression pattern in renal glomeruli of diabetic mice compared with non-diabetic mice. Some of these microRNAs are known to be related to inflammatory process and cell proliferation. Altered expression of these microRNAs may be associated with pathogenesis of diabetic nephropathy.
Supported by: Scientific Research from the Ministry of Education, Science, Culture, Sport

OP 19 Large studies - new data

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Estimating the quality of life impact of diabetes related complications: new results from the UKPDS
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Background and aims: Reliable estimates of the quality of life impact of diabetes related complications are important for researchers conducting trial-based and model-based evaluations of the cost-effectiveness of interventions. Previous estimates based on a cross-sectional study of patients enrolled in the UKPDS have been widely used. Here we report updated results drawing on the UKPDS Post Trial Monitoring Study, which allows us to greatly extend follow-up, examine a larger number and wider range of complications, and compare cross-sectional results with those from repeated measures of quality of life over time.
Materials and methods: Quality of life was measured using the EuroQol EQ-5D instrument, which was administered in 1996/7 and again annually over the period 2002-8 to all remaining participants in the study. We estimated the immediate and long term impact on quality of life of myocardial infarction, ischaemic heart disease, stroke, heart failure, amputation, renal failure, blindness in one eye, retinal photocoagulation, cataract extraction and vitreous haemorrhage, controlling for age, sex and diabetes duration. We also compared different methods of estimating these effects.
Results: A total of 4267 UKPDS patients were administered one or more EQ-5D questionnaires. The average number of questionnaires completed was 3.4 and the maximum was 7. Approximately 49% of respondents only answered the questionnaire once. 1425 patients died between 1997 and 2008. The response rate of fully completed questionnaires varied from 68% to 74%. Compared with our original study, many more complications were available for analysis: for example, in our data set the total number of myocardial infarctions increased from 203 in the first questionnaire to 371 across all rounds, the number of amputations from 21 to 89, blindness to one eye from 93 to 197 and stroke from 60 to 171. Mean quality of life was seen to decline from 0.77 at the first questionnaire in 1996/7 to 0.64 in the last questionnaire in 2007/8. This was related to the increasing age of patients over time (62 at first quality of life questionnaire compared with 72 at final questionnaire), and an increasing proportion of patients with a history of complications (27% at first questionnaire compared with 55% at last questionnaire). For those without complications of any sort the average utility across questionnaires was 0.74, compared with 0.66 for those with a history of myocardial infarction, 0.46 with amputation, 0.60 with blindness to 1 eye and 0.53 with stroke.
Conclusion: This paper reports new results from the UKPDS on the quality of life impact of a range of diabetes related complications. These suggest that complications have substantial and long-lasting effects on patient quality of life. The results are based on well-validated patient history and adjudicated complications data from a landmark diabetes study, and should be useful in estimating more accurately the outcome of interventions that reduce these complications.
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The majority of type 2 diabetic patients with renal impairment have non-albuminuric renal disease - the Swedish National Diabetes register (NDR)
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Background and aims: Albuminuria and renal impairment are two main manifestations of renal disease which are not entirely linked in patients with type 2 diabetes (T2D). The aim of this cross-sectional study was to identify the prevalence of non-albuminuric renal impairment in type 2 diabetic patients and to examine the clinical characteristics associated with non-albu-

minuric renal impairment in a large nation-wide population-based diabetes register.

Materials and methods: 62 661 patients with T2D aged 30-80 years with complete datasets on albumin excretion, renal function (serum creatinine) and clinical characteristics reported to the Swedish National Diabetes Register in 2008 were included. Albuminuria was defined as urinary albumin excretion rate > 20 μg/min and renal impairment as estimated glomerular filtration rate; eGFR< 60 ml/min/1.73 m^2 according to MDRD. Logistic regression analyses for clinical and biochemical variables with renal impairment with or without albuminuria as dependent variable were performed. Adjusted odds ratios were calculated and continuous variables were increased per one standard deviation. 95% confidence intervals are given.

Results: 15% of all patients had renal impairment (n=9 308). Among patients with renal impairment 56% were non-albuminuric and 42% were albuminuric. In a multivariate analyses patients with non-albuminuric renal impairment had significantly and independently shorter diabetes duration (adj OR 0.73; 95% CI 0.70-0.76), higher total cholesterol (1.05; 1.01-1.10), lower levels of triglycerides (0.83; 0.80-0.87), lower systolic blood pressure (0.81; 0.78-0.84), better glycemic control (HbA1c%) (0.86; 0.82-0.91), lower BMI (0.88; 0.84-0.93) and were more often female and non-smoking as compared with patients with albuminuric renal impairment.

Conclusion: The majority of patients with type 2 diabetes and renal impairment have non-albuminuric renal disease. Distinct sets of risk factors were associated with the presence or absence of albuminuria, patients with non-albuminuric renal impairment exhibiting less features of the "metabolic syndrome". Non-albuminuric renal impairment could partly be explained by the use of renin angiotensin system inhibitors but our results also support the concept of different underlying pathophysiology mechanisms. Development of markers and methods for a more accurate estimation of renal function in T2D patients without albuminuria is important for screening, follow-up and treatment of these patients.

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Risk of progression of nephropathy in a population-based sample with type 2 diabetes

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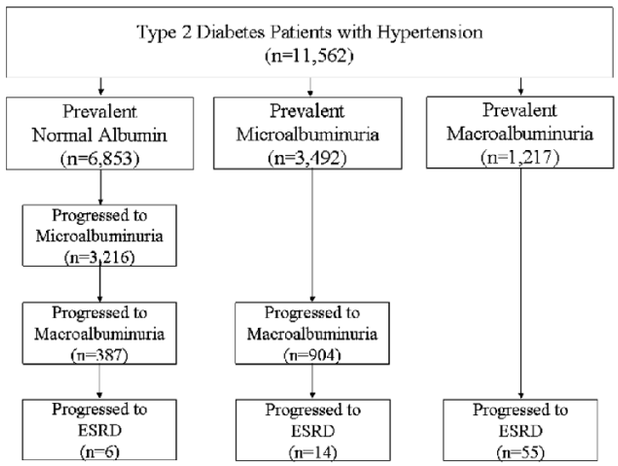
Background and aims: Progression through stages of nephropathy has not been well described in a large, well-characterized, population-based study. Estimates of the public health burden of nephropathy may be different in such a population. Our aims were to describe the progression of nephropathy and identify demographic and clinical characteristics associated with progression in a U.S. population-based sample.

Materials and methods: We identified 11,562 members of a managed care organization who had hypertension and type 2 diabetes, a urine albumin-to-creatinine ratio (UACR) measurement in 2001-2003, and at least 1 follow-up UACR. Baseline nephropathy stage was defined as normal albumin (UACR<3.4 mg/mmol), microalbuminuria (3.4-33.9 mg/mmol), and macroalbuminuria (≥33.9 mg/mmol). We searched records through 2008 for progression from baseline to a higher stage of nephropathy including ESRD.

Results: Mean age was 59.4±11.3 years; 49.8% were male, 17.5% African-American, and mean A1C was 8.1%. At baseline, 59% had normal albumin, 30% had microalbuminuria, and 11% had macroalbuminuria. The incidence of nephropathy progression (per 1000 person-yrs) was 94.6, 44.1, and 6.7 for normal albumin, micro-, and macro- albuminuria, respectively. The high rates of progression of nephropathy among those with normal albumin demonstrate that 68% of all patients had developed micro- or macro- albuminuria by the end of follow-up. Most patients received antihypertensive therapy; ACEi/ARB use ranged from 61-67%, except among patients with macroalbuminuria at follow-up. Age, diabetes duration, and A1C were significant predictors of progression.

Conclusion: Our study, one of the first to examine the progression of nephropathy in a U.S population-based sample, showed that among adults with diabetes and hypertension, the lifetime risk of nephropathy and it's progression may be greater than previously reported. Further, the use of ACEi/ARBs to slow and/or prevent the progression of nephropathy may be underutilized.

Figure. Prevalence of Nephropathy and Progression to Subsequent Stages



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Prospective association of B-type natriuretic peptide with left ventricular systolic and diastolic function in individuals with and without type 2 diabetes - the Hoorn Study

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Background and aims: Individuals with type 2 diabetes mellitus (T2DM) have an increased risk of developing heart failure (HF). Higher plasma B-type natriuretic peptide (BNP) in a non-heart failure range predicts HF and CVD mortality and is associated with T2DM. We aimed to investigate prospectively in a population based cohort the association of BNP levels in a non heart failure range with left ventricular (LV) mass, LV systolic function, and LV diastolic function in individuals with and without T2DM.

Materials and methods: In the Hoorn Study, a population-based prospective cohort study, plasma BNP (pmol/l) was determined at baseline. A 2D echocardiogram was made at baseline and after 8 years of follow-up to measure LV mass index (LVMI, g/m^2), ejection fraction (EF, %, systolic function) and left atrial volume index (LAVI, ml/m^2, diastolic function). Participants with atrial fibrillation, wall movement abnormalities and moderate or severe aortic or mitral valve disease were excluded. Linear regression analyses, adjusted for gender, age, baseline heart function, use of antihypertensive medication, BMI and heart rate were performed to investigate the association of BNP with LVMI, and LV systolic and diastolic function. In case of significant effect modification ($p<0.10$), we reported the regression coefficients for individuals with and without T2DM separately.

Results: Of the 796 individuals of whom echocardiograms at baseline were present, 441 (55%) attended the follow-up examination (baseline age 66 years, 34% T2DM). Increase in LVMI was greater in those with higher baseline BNP (Table 1). The association was stronger in patients with T2DM, and in non-T2DM the association was explained by baseline LVMI, BMI and use of antihypertensives, in T2DM the association was independent. Regardless of T2DM, a 10 pmol/l higher baseline BNP was associated with a 2.7% lower EF and a 5.0 ml/m^2 higher LAVI at follow-up.

Conclusion: This study shows that BNP levels are prospectively associated with LV diastolic and systolic function and that there is a strong association between BNP levels in a non-heart failure range and LVMI for individuals with T2DM and not for those without.

Table 1: Coefficients (95% CI) per 10 pmol increase of baseline BNP for LVMI, EF and LAVI (follow-up)

LVMI (g/m2)	**non-T2DM** n = 161	**T2DM** n = 86
Crude model	5.7 (-0.03 - 11.5)	25.1 (11.0 - 39.3)*
adjusted for gender and age	6.5 (1.0 - 11.9)*	26.3 (11.4 - 41.2)*
+ adjusted for baseline LVMI, antihypertensives, BMI and heart rate	1.1 (-4.6 - 6.8)	29.1 (13.6 - 44.6)*
EF (%, LV systolic function)	Total population n = 235	
Crude model	-2.3 (-4.4 - - 0.1)*	
adjusted for gender and age	-2.3 (-4.5 - - 0.1)*	
+ adjusted for baseline EF, antihypertensives, BMI and heart rate	**-2.7** (-4.9 - - 0.4)*	
LAVI (ml/m2, LV diastolic function)	n = 268	
Crude model	7.3 (5.3 - 9.2)*	
adjusted for gender and age	7.0 (5.1 - 9.0)*	
+ adjusted for baseline LAVI, antihypertensives, BMI and heart rate	5.0 (2.9 - 7.1)*	

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Arterial stiffness is prospectively associated with left ventricular diastolic function in individuals with and without type 2 diabetes - The Hoorn Study

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Background and aims: Left-sided heart failure (HF), especially with a normal ejection fraction, is common with type 2 diabetes (T2DM), but the underlying mechanisms remain controversial. Arterial stiffness is suggested as a potential cause of HF. We investigated whether arterial stiffness was prospectively associated with a higher left ventricular (LV) mass and worse LV diastolic function and whether this differed in individuals with or without T2DM.
Materials and methods: In the Hoorn Study, a population-based cohort study of diabetes, echocardiography and arterial ultrasonography was performed in 2000 and 2008. Linear regression analyses were performed to investigate associations of carotid, brachial and femoral artery distensibility coefficients (DC, arterial stiffness) with LV mass index (LVMI, g/m^2) and left atrial volume index (LAVI, ml/m^2, LV diastolic function). Individuals with moderate or severe mitral or aortic valve disease, or tachycardia (heart rate>90bpm) were excluded. Analyses were adjusted for age, sex, BMI, and baseline value of the outcome. Influence of T2DM or medication use was investigated by stratified analyses.
Results: Of the 796 individuals of whom echocardiograms at baseline were present, 441 (baseline age 66 years, 34% T2DM of whom 80% were newly diagnosed) attended the follow-up. Non-attendees were older, had lower DCs and higher LVMI and LAVI as compared to attendees. In crude analyses, femoral and carotid DC were significantly associated with LVMI, however this was explained by age and sex differences. Associations between DCs and LAVI were not different for individuals with or without T2DM (p for interaction >0.10). Individuals with T2DM had at baseline already lower DCs and higher LAVI compared to those without T2DM. In individuals who did not use lipid or glucose lowering medication, every 10^{-3} kPa^{-1} lower baseline brachial and femoral DC was independently associated with a 0.41 (95% CI: 0.14-0.67) or 0.68 (0.12-1.23) ml/m^2 higher LAVI, respectively. Associations adjusted for mean arterial pressure were 0.36 (0.09-0.63) and 0.57 (-0.01-1.14, NS) ml/m^2 higher LAVI per 10^{-3} kPa^{-1} lower DCs. The associations were absent in those who used glucose and/or lipid lowering medication. Further adjustments for HbA_{1c}, heart rate, LVMI, systolic BP or use of antihypertensive medication did not change our results.
Conclusion: Arterial stiffness was prospectively associated with a worse LV diastolic function, regardless of T2DM. These associations were only partly explained by mean arterial pressure. Because individuals with T2DM commonly have stiffer arteries than those without T2DM, our finding indicates that arterial stiffening might be one of the causes of a worse LV diastolic function in individuals with T2DM.
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Natural course of glucose effectiveness in nondiabetic and diabetic individuals: the Insulin Resistance Atherosclerosis Study

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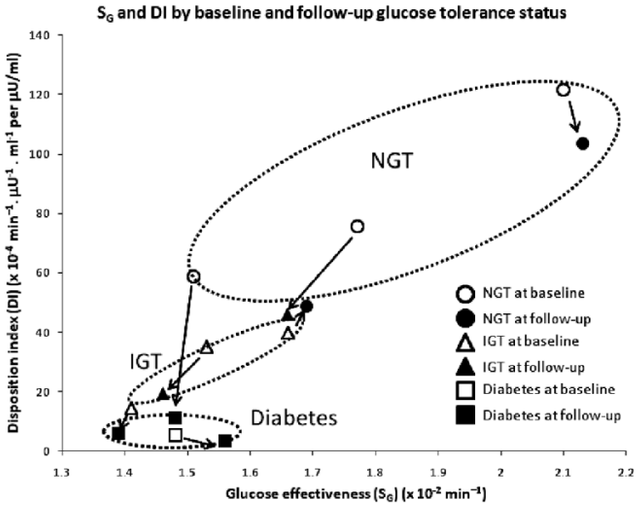
Background and aims: Disposition index (DI), the product of insulin sensitivity and secretion, decreases as glucose tolerance status deteriorates. Glucose effectiveness (S_G), the ability of glucose to enhance its own cellular uptake and to suppress endogenous glucose production, is also a risk factor for type 2 diabetes, but the natural course of S_G is not well characterized. Thus, we examined the natural course of S_G relative to that of DI in participants in the Insulin Resistance Atherosclerosis Study (IRAS).
Materials and methods: DI and S_G were measured in 923 IRAS participants aged 40 - 69 years (Hispanics, non-Hispanic whites, and African Americans) by the frequently sampled intravenous glucose tolerance test. DI and S_G were also measured using the same protocol at the 5-year follow-up examination. DI was expressed as the product of insulin sensitivity index and acute insulin response. Normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and diabetes were defined by the 1999 World Health Organization criteria. Individuals treated with glucose-lowering medications were excluded.
Results: At baseline, S_G was 1.97 ± 0.03 in individuals with NGT, 1.53 ± 0.05 in those with IGT, and 1.48 ± 0.03 x 10^{-2} min^{-1} in diabetic participants (p_{trend} <0.001). Corresponding values of DI were 103.6 ± 5.4, 27.8 ± 2.4, and 5.2 ± 0.6 x 10^{-4} min^{-1}. μU^{-1}. ml^{-1} per μU/ml (p_{trend} <0.001). S_G was directly related to DI (r = 0.40, p <0.001) by the Spearman's rank test after controlling for age, sex, race/ethnicity, and center. During the follow-up period, DI decreased in all glucose tolerance categories except in individuals who had IGT at baseline and NGT at follow-up (Figure). In individuals with NGT at baseline, the decrease in DI (adjusted for baseline DI) was directly related to worsening of glucose tolerance status during the follow-up period (p_{trend} <0.001). Similar results were observed in individuals with IGT at baseline (p_{trend} <0.001). S_G also decreased with time in all categories but in participants with NGT who had no change of status and in those with IGT whose status improved. In individuals with NGT at baseline, the decrease of S_G (adjusted for baseline S_G) was directly related to worsening of glucose tolerance status during the follow-up period (p_{trend} <0.001); however, results were not statistically significant in those with IGT at baseline (p_{trend} = 0.075).
Conclusion: Failure to maintain DI and S_G results in worsening of glucose tolerance status.



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OP 20 Diabetic foot - mechanisms and treatment

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RANKL/OPG signalling pathway mediates medial arterial calcification in diabetic Charcot neuroarthropathy

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Background and aims: Patients with Charcot neuroarthropathy (CN), display a paradoxical coexistence of medial arterial calcification (MAC) and osteolysis, with the suggestion of a key role for RANKL/OPG signal modulation. We aimed to study the potential mechanisms of RANKL/OPG-mediated vascular calcification in CN.
Materials and methods: Serum was obtained from 12 patients with acute CN, 10 patients with diabetes and 5 non-diabetic controls. Arterial segments were obtained from patients undergoing lower limb amputation and were fixed, sectioned and stained with Alizarin red and primary human RANK-L antibody. Human vascular smooth muscle cells (VSMCs) were explanted from the same arterial segments for subsequent cell culture experiments.
Results: Using Bioplex multi-array technology and ELISA bioassays, we demonstrated: i), higher serum OPG (8.2±2.7 vs 7.7±3.3 vs 4.2±0.4pM, p=0.031); ii), higher RANKL/OPG ratio (36.8±43.1 vs 5.2±4.9 vs 4.6±3.1 pM/pM, p= 0.033); iii), higher inflammatory cytokines IL-8 (p<0.0001) and G-CSF (p=0.002); and iv), a trend towards higher RANKL (0.32±0.42 vs 0.04±0.05 vs 0.02±0.01pM, p=0.054) in CN compared to diabetic and non-diabetic controls respectively. Cultured VSMCs displayed accelerated osteoblastic differentiation confirmed by Alkaline phosphatase activity (μM phosphate/mg protein/min) in the presence of serum from CN patients(49.9±7.3μM) compared to diabetic serum (29.8±3.7μM, p=0.012), control serum (17.6±10.1, p=0.011) or osteogenic medium (OM) controls (15.3±5.4μM, p=0.003). Cultured VSMCs also displayed increased mineralisation after Alizarin red staining and dye elution in Charcot serum (OD units, 0.16±0.01, p=0.0003); diabetes serum (0.15±0.01, p=0.0004); and marginally higher in 'Healthy' serum (0.14±0.03, p=0.04) compared to OM controls (0.09±0.003). Mineralisation and differentiation of VSMCs induced by Charcot serum were significantly attenuated (p=0.018 and p=0.004 respectively) when co-incubated with OPG, the decoy receptor for RANKL signalling. Immunohistochemical analysis of arterial sections showed positive RANKL expression within the vicinity of MAC compared to control non-calcified sections.
Conclusion: These data demonstrate that in humans, RANKL/OPG signalling is modulated and implicated in MAC and in CN, thus suggesting that anti-RANKL therapy may be therapeutic in acute CN or could have potential preventative value in recurrent CN.

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Role of osteoprotegerin (OPG) in predicting development of foot ulcer and loss of foot pulse in type 1 diabetic patients with and without diabetic nephropathy

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Background and aims: The bone-related peptide osteoprotegerin is produced by vascular cells and is involved in the process of vascular calcification previously shown to predict mortality and cardiovascular events. We investigated the predictive value of plasma OPG in relation to foot complications in patients with type 1 diabetes (T1DM) with and without diabetic nephropathy.
Materials and methods: Prospective observational follow-up study of 397 type 1 diabetic patients with overt diabetic nephropathy (243 men; age [mean ± SD] 42.1 ± 10.6 years, duration of diabetes 28.3 ± 9.9 years, GFR 67 ± 28 ml/min/1.73 m^2) and a control group of 176 patients with longstanding type 1 diabetes and persistent normoalbuminuria (105 men; age 42.6 ± 9.7 years, duration of diabetes 27.6 ± 8.3 years). p-OPG was measured by ELISA.
Results: During the 12 (0-15) years (median (range)) of follow-up, 107 (40%) with OPG levels above the median *vs.* 76 (27%) below developed a foot ulcer,

p=0.001. This corresponds to a hazard ratio (HR) of 1.7 [1.3-2.3] and covariate adjusted (sex, age, nephropathy status, smoking, HbA_{1c}, systolic BP, eGFR, and CRP) HR 1.5 [1.0-2.1], p=0.04. Furthermore, 42 (16%) patients with higher OPG *vs.* 15 (5%) with lower lost foot pulse, adj. HR 2.2 [1.0-4.6], p=0.04. Similarly, 51 (18%) with higher OPG levels *vs*. 21 (7%) with lower had vascular surgery or amputation performed, HR 2.9 [1.7-5.0], p<0.001, however after adjustment this was no longer significant (p=0.53). In contrast, OPG levels were not related to loss of vibration perception or development of a Charcot foot, (p=0.11 and 0.27, respectively).
Conclusion: OPG is elevated in T1DM patients who developed a foot ulcer, lost foot pulse or had vascular surgery or amputation performed during follow-up. Furthermore, higher OPG levels remained an independent predictor of development of foot ulcer and lost foot pulse when adjusting for covariates.
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The diabetic foot in Germany: analysis of quality in specialised diabetic footcare centres
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Background and aims: The German Working Group on the Diabetic Foot developed certification requirements for diabetic foot centres. These certification requirements established procedures by which specialised centres for the treatment of the diabetic foot syndrome could verify their management quality. In addition, this certification fulfils the demands of the 2006 IDF Global Guideline for Type 2 Diabetes. The aim was to establish comparable diabetic foot centers with clearly defined treatment structures.
Materials and methods: Conditions for the certification are quality parameters of the facility's structure, treatment procedures and patient outcomes. Structural quality was based on the qualifications of staff, the facility's spatial conditions and a minimum provision of equipment. Staff members of certified centres must visit each other. Also assessed are the application of available guidelines and documentation systems, the establishment of a multidisciplinary team approach between the facility's staff and other experts involved. For the evaluation, each centre documented 30 consecutively seen individuals with diabetic foot lesions. An evaluation of the outcomes was performed 6 months after the initial presentation. All parameters are checked, presented and benchmarked in an open session of the working group.
Results: Data from 428 certified and re-certified centres are presented and a total of 12606 individuals were evaluated. At the 6-month follow-up assessment more than half of the lesions were completely healed (58%). The results of the evaluation indicate a low level of major (above-ankle) amputations compared with the expected amputation rates in Germany (nearly 10-15%).

Data given in %	Major-Amputation	Minor-Amputation	died
2005	3,9	19	4,6
2006	3,9	24	5,4
2007	3,6	20,1	5,1
2008	2,9	18,8	4,7
2009	2,7	16,7	2,9

Conclusion: These data represent the first analyses of the treatment outcomes of diabetic foot lesions in specialised centers in Germany over a time period of 5 Years. The data reflect a lower rate of major and minor amputations in specialist centres compared with the available epidemiological data for Germany as a whole. This analysis presents the first German data collected using defined standards that include amputation rate and mortality in the treatment of the diabetic foot in specialist centres. The established structures are appropriate to show consistent low rates of amputations over several years.
Supported by: Working Group of the Diabetic foot of the German Diabetes Association

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Acute antimicrobial effect of maggot therapy on diabetic foot ulcer infection as a basis for identification of antimicrobial peptides from maggots
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Background and aims: The treatment of infected diabetic foot ulcers (DFU) is often complicated by emergence of antibiotic resistance; maggot debridement therapy (MDT) may be helpful in overcoming this problem. Antimicrobial effect of MDT and stimulation of immune response were proven in vitro. The aim of our study was to assess the antimicrobial effect of MDT on different strains of bacteria in a 5-year cohort of patients with infected DFU as a basis for identification of antimicrobial peptides from maggots.
Materials and methods: 91 patients with infected DFU treated in our diabetic foot clinic were enrolled in the present study between January 2005 and February 2010. Sterile free-range larvae of the green bottle fly Lucilia sericata were applied to the ulcers for 3-5 days (5-10 larvae per cm^2). Swabs or tissue samples were taken from deep structures of the wound after debridement. Specimens for culture were obtained immediately before and after MDT; control specimens were taken 10 (±3) days after the end of MDT. The individual bacterial species in positive swabs were determined by standard microbiological methods and microorganisms were categorized by Gram staining. MRSA was identified according to positive test for mec gene. High-performance liquid chromatography, mass spectrometry and Edman degradation were used for identification and isolation of antimicrobial peptides.
Results: The mean duration of MDT was 3.6±0.4 days. There was a significant elimination of majority of bacteria immediately after MDT: MRSA (21/91 vs. 5/91; p<0.001], Enterococcus sp. (36/91 vs. 15/91; p<0.001), Staphylococcus coagulase negative (21/91 vs. 7/91; p<0.001), Streptococcus sp. (6/91 vs. 0/91; p<0.001), Escherichia coli (13/91 vs. 5/91; p<0.02), Proteus sp. (13/91 vs. 5/91; p<0.01) and Klebsiella sp. (12/91 vs. 5/91; p<0.01). Antimicrobial effect persisted 10±3 days after cessation of MDT for all these strains of bacteria (all p<0.02). MDT was ineffective against Pseudomonas sp. (12/91 vs. 9/91; NS) and Acinetobacter sp. (6/91 vs. 4/91; NS). Antimicrobial peptide named lucifensin was identified from maggots after MDT of infected DFU and the primary sequence of this peptide was determined. In vitro, lucifensin was effective against Staphylococcus aureus PS 3A, PS 77, methicilin-resistant Staphylococcus aureus, Baccilus subtilis, Rhodococcus sp., but was ineffective against Esherichia colli and Pseudomonas aeruginosa. There was some correspondence between in vitro assessed antimicrobial effect of lucifensin and clinical effect of MDT.
Conclusion: Our study demonstrated that MDT acutely eliminated most of the Gram-positive including MRSA and Gram-negative strains in patients with infected DFU, but was ineffective against Pseudomonas sp. and Acinetobacter sp. The insect defensin designated lucifensin was isolated from maggots as a promising antimicrobial peptide.
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A double blind randomised controlled trial of the efficacy of soluble beta-1,3/1,6-glucan in the management of chronic foot ulcers in diabetes
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Background and aims: Beta-glucans from a variety of non-animal sources have been shown to have immuno-modulatory effects in animals and humans, partly by being recognised by the Dectin-1 receptor and partly by a specific interaction with complement receptor 3 - both of which are present on mammalian phagocytic cells, including neutrophils and macrophages. It is therefore possible that the administration of a beta-glucan such as soluble beta-1,3/1,6-glucan (SBG) might improve the healing of chronic wounds in diabetes through such an action. Since an earlier small open label pilot study and a double blind phase II study of SBG had suggested benefit, this trial was designed as a Phase III study.
Materials and methods: Patients with diabetes and full-thickness foot ulcers present for >4 weeks but less than 2 years, and of area between 25mm^2 and 500 mm^2, were randomised 1:1 to have either 2% aqueous SBG applied or

matching methyl cellulose placebo applied to their wounds on two or more occasions each week for 8 weeks. Those with active infection, without palpable foot pulses or with ABPI <0.7 were excluded. The primary outcome measure was healing within 8 weeks. Secondary outcomes included time to healing, breakdown of healed ulcers within 12 weeks, change in ulcer area, safety, patient well-being and satisfaction (EQ-5D, Cardiff Wound Impact Schedule).
Results: 122 patients (mean age 58.5y; 76.2% male) in 10 UK centres were randomised with 67 (54.9%) in the SBG group; the demographic and baseline characteristics of the two groups were comparable. Eight (3 SBG, 5 placebo) withdrew because of adverse event (5), death (1) or protocol violation (2). There was no difference in numbers healing within 8 weeks in the two groups (31.3% SBG versus 32.7% placebo; ITT p=0.87 CMH test). There was similarly no difference in time to healing in those that healed (ITT p=0.84, stratified log rank), or in change in ulcer area (ANCOVA p=0.45). There was no difference in change in local pain, or in EQ-5D and CWIS scores. 17.9% ulcers recurred within 12 weeks of complete healing, but there was no difference between groups (p=0.062 CMH). The majority of adverse events were mild or moderate in severity and unrelated to treatment.
Conclusion: We were unable to confirm the superiority of SBG versus placebo in terms of both primary and secondary endpoints in this study.

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Comparison of healing of ischaemic diabetic foot ulcers after stem cell therapy or percutaneous transluminal angioplasty
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Background and aims: Critical limb ischaemia (CLI) is an important prognostic factor for ulcer healing in patients with diabetic foot ulcers (DFU) and often leads to amputations. Treatment of CLI in diabetic patients is generally difficult. Autologous stem cell therapy is a new therapeutic method for patients with CLI while the standard therapy remains percutaneous transluminal angioplasty (PTA) or peripheral vascular by-pass. The aim of our study was to compare healing of DFU in patients treated by stem cell therapy and PTA.
Materials and methods: Ten patients treated by stem cells at our foot clinic between January 2008 and October 2009 were included into the study (SCT group). All patients were diagnosed of CLI (defined by transcutaneous oxygen tension [$TcPO_2$] < 30 mm Hg), ulcer size was between 0.5 and 7 cm^2 with Texas classification 1C and 2C and no signs of acute infection. Stem cell therapy was indicated in patients with persisting ischaemia after standard revascularization. Autologous stem cells were obtained from bone marrow or peripheral blood after stimulation by growth factor and applied into muscles of affected lower limb. Ten patients from our foot clinic with the same inclusion criteria treated by angioplasty (PTA group) during the same period were included into the control group. They did not significantly differ from SCT group in mean age (62 ± 10 vs. 64 ± 8.5 years), gender (80 % vs. 70 % men), glycated hemoglobin (7.4 ± 0.8 vs. 7.6 ± 1.2 %) and mean diabetes duration (25 ± 11 vs. 20 ± 7.1 years). All patients were treated by standard podiatric methods. Area defect reduction was assessed after 3 and 6 months and time to full healing was evaluated 6 months after stem cell therapy or PTA.
Results: There was no significant difference in defect area (2.6 ± 1.5 vs. 2.7 ± 2.5 cm^2) and $TcPO_2$ (16.3 vs. 14.3 mm Hg) between both groups before the treatment. Area defect reduction was significantly faster in SCT group in comparison with PTA group after 3 months (20 ± 89.8 vs. 84.1 ± 30.3 %, p = 0.05); reduction after 6 months was not significantly different (38.1 ± 109.4 vs. 93.2 ± 13.3 %, NS). Six months after the therapy were healed 6/10 patients in both groups, but time to full healing was significantly shorter in SCT group (65.8 ± 20.1 vs. 126 ± 39 days, p = 0.016).
Conclusion: Our study showed faster healing of DFU after stem cell therapy than after PTA in patients with CLI and the same effect on healed patients after 6 months. Autologous stem cell therapy is a promising alternative for treatment of severe ischemia and DFU, but randomized controlled studies are required to confirm these results.
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OP 21 Intertissue crosstalk in metabolism

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Selective elevation of insulin in the head suppresses hepatic glucoregulatory gene expression and net hepatic glycogenolysis in the conscious dog
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Background and aims: Studies in the rodent have demonstrated that hypothalamic insulin signaling can play a role in controlling hepatic glucose production (HGP). However, we previously found in the dog that blockade of insulin signaling in the brain did not impair the suppression of HGP during a physiological elevation of insulin throughout the body. Thus, brain insulin signaling may not regulate HGP in non-rodent species with lower basal HGP, or alternatively, the effect of hepatic hyperinsulinemia may mask the subtle effects of brain insulin action. To determine the degree to which insulin action in the brain might contribute to inhibition of HGP in the presence of basal insulin and glucagon levels in the dog, insulin was selectively elevated in the blood perfusing the brain.
Materials and methods: Dogs underwent head (carotid and vertebral artery; jugular vein) and liver (femoral artery; portal and hepatic vein) catheterization and a cannula was inserted into the 3rd ventricle (ICV). On the day of the study, at -150 min [3-^{3}H]glucose and somatostatin (to inhibit pancreatic hormone secretion) were infused into a peripheral vein and glucagon and insulin were infused intraportally at basal rates. Following a basal sampling period (-30 to 0 min), artificial cerebrospinal fluid (aCSF; n=7) or the PI3K inhibitor LY29004 (to block brain insulin action; LY; n=8) was infused ICV (0 to 300 min). From 60 to 300 min, the liver insulin level remained clamped at basal while plasma insulin was selectively increased (10-fold) in the head by infusion of the hormone into the carotid and vertebral arteries, coincident with a cessation of portal insulin infusion. Intralipid, heparin and glucose were infused as required to clamp NEFA and glucose at basal.
Results: In both groups head insulin (jugular) increased 10-fold, arterial insulin increased 2-fold, and insulin at the liver remained basal. Glucose, glucagon and NEFA remained at basal levels. The change in net hepatic glucose balance between basal and the last hour in the LY and aCSF groups, respectively, was -0.09±0.12 and -0.58±0.27 mg/kg/min (P=0.05). The change in endogenous glucose Ra was -0.17±0.12 and -0.35±0.12 mg/kg/min, respectively. Net hepatic glycogenolytic (NHGLY) and gluconeogenic flux (GNG) were estimated using net liver balance. Delta NHGLY was -0.15±0.07 and -0.63±0.23 mg/kg/min (P<0.05) in the two groups, respectively, while delta GNG was 0.06±0.11 and 0.00±0.08 mg/kg/min. Liver P-Akt was basal in both groups at the end of the study, while hypothalamic P-Akt was basal in LY but had increased 2-fold in aCSF (P<0.05). Likewise, hepatic P-STAT3, reported to be increased by brain insulin signaling, was basal in LY but had increased 40% in aCSF (P<0.05). Liver PEPCK gene expression was reduced by 50% when insulin was selectively elevated in the head.
Conclusion: In the absence of an increase in insulin's direct hepatic effects, insulin signaling in the brain affected gene expression in the liver in a large animal model. HGP tended to be reduced although the effect was small, did not become significant until 2.5 h, and was due to inhibition of net hepatic glycogenolysis. It would appear that the rapid decrease in HGP which normally occurs in response to an increase in insulin secretion in vivo is unrelated to brain insulin signaling.
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MCP-1 and chemerin are involved in the regulation of anandamide secretion by human skeletal muscle cells
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Background and aims: Obesity is one of the major components of the metabolic syndrome and associated with increased adipose tissue mass, which is characterised by an altered secretion profile. It has been shown that be-

sides other factors the levels of proinflammatory cytokines like MCP-1 and chemerin are elevated, and their implication in the induction of insulin resistance in skeletal muscle could be demonstrated. In addition, obesity is associated with a dysregulation of the endocannabinoid system leading to elevated plasma levels of anandamide (AEA) and 2-arachidonoylglycerol (2-AG) as well as decreased expression and activity of the AEA-degrading enzyme fatty acid amide hydrolase (FAAH). Previously, we could show that AEA is involved in the induction of skeletal muscle insulin resistance via enhanced IRS-1(Ser307) phosphorylation. Aim of the present study was to investigate whether skeletal muscle cells themselves are able to produce endocannabinoids and whether their secretion is regulated by selected cytokines.
Materials and methods: Differentiated primary human skeletal muscle cells (SkM) were incubated with 0.5 µg/ml chemerin and 2 ng/ml MCP-1, respectively. Medium was collected after 24 h, endocannabinoids were extracted and analysed by liquid chromatography/mass spectrometry. Additionally, RNA was prepared after 8 h or 24 h incubation and used for qRT-PCR to study the expression of FAAH and the AEA-synthesizing enzyme N-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD).
Results: In media obtained from 3 different SkM donors we were able to detect AEA and 2-AG as well as palmitoyl ethanolamide (PEA) and oleoyl ethanolamide (OEA). The concentration in media from untreated cells was 1.75±0.08 pmol/l for AEA, 260.06±35.99 pmol/l for 2-AG, 6.38±2.58 pmol/l for PEA and 3.09±0.32 pmol/l for OEA. Stimulation of SkM with MCP-1 and chemerin for 24 h led to a significant increase of AEA, while for 2-AG, PEA, and OEA no significant changes were observed. MCP-1 treatment resulted in a 1.3-fold and chemerin treatment in a 1.8-fold increase of AEA concentration (n=3). The analysis of NAPE-PLD and FAAH expression showed that after 8 h incubation with MCP-1 the expression of NAPE-PLD was increased by ~15% and with chemerin by ~20%, while no change in FAAH expression was observed. However, after 24 h the expression of FAAH was downregulated by MCP-1 (~16%) and chemerin (~26%), while the expression of NAPE-PLD was at the level of untreated controls (n=3).
Conclusion: To the best of our knowledge, these results demonstrate for the first time the ability of human skeletal muscle cells to produce and secrete endocannabinoids such as AEA and 2-AG. In addition, we could prove that the proinflammatory cytokines MCP-1 and chemerin are involved in the regulation of AEA synthesis by causing an increase of AEA level. Part of this regulation includes changes of NAPE-PLD and FAAH expression. In summary, our data link MCP-1 and chemerin, which are known to induce skeletal muscle insulin resistance by themselves, to the endocannabinoid system and AEA, which also has been shown to play a role in the induction of skeletal muscle insulin resistance. In states of obesity, which are characterised by elevated levels of proinflammatory cytokines and AEA, the influence of MCP-1 and chemerin on AEA synthesis in muscle tissue may thus lead to further impairment of insulin sensitivity.

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Secreted adipocyte fatty acid binding protein (FABP4) forms the molecular basis of the adipo-insular axis
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Background and aims: Obesity is closely linked with increased insulin levels and β-cell mass. Obesity may be accompanied by adipose tissue hypoxia, which occurs when the rate of adipose tissue expansion exceeds the rate of required angiogenesis, leading to areas that are insufficiently vascularised and consequently hypoxic. We hypothesised the link between obesity and insulin levels to be a result of an inter-organ communication between hypoxic adipose tissue and the β cell, which may involve a secretory component.
Materials and methods: To assess the effects of adipocyte conditioned media on β-cell function, 3T3-L1 adipocytes were incubated in serum free culture media (RPMI1640) under atmospheric or 1% O2 concentrations. Conditioned media was dialysed and concentrated against a 10 kDa filter to remove small non-protein molecules. Dialysed conditioned media was diluted with 10% foetal calf serum and used to treat islets isolated from mouse pancreas for 24 hr. Glucose stimulated insulin secretion from islets was subsequently assayed. A stable isotope labelling of amino acids in cell culture (SILAC) quantitative mass spectrometry screen was performed to identify hypoxia regulated adipocyte secretory proteins.
Results: Conditioned media from hypoxic, but not normoxic control 3T3-L1 adipocytes led to an increase in glucose stimulated insulin secretion of 70% (412.7 ± 83.03 vs. 753.5 ± 108.6 pg insulin / islet / hr, p<0.05). A quantitative mass spectrometry screen led to identification of the adipocyte fatty acid binding protein FABP4 as a hypoxia responsive adipocyte secretory protein. Western blotting and ELISA data confirmed increased secretion of FABP4 from adipocytes into conditioned media during hypoxia (146.9 ± 16.64 vs. 1166 ± 61.18 ng / ml FABP4, p<0.0001). Serum concentrations of FABP4 were raised in high fat fed mice, along with circulating insulin concentrations (chow fed 259.9 ± 67.05 vs. high fat fed 804.8 ± 173.7 ng / ml serum FABP4, p<0.05; 1.397±0.470 vs. 3.205±0.549 ng / ml serum insulin, p<0.05). In vitro treatment of islets with physiologically relevant concentrations of recombinant FABP4 pre-incubated with linoleate but not palmitate enhanced glucose stimulated insulin secretion to a degree similar to hypoxic adipocyte conditioned media (535.4 ± 32.32 vs. 947.0 ± 134.5 pg insulin / hr, p<0.05). Hypoxia inhibits mitochondrial function, and treatment of adipocytes with the mitochondrial poisons DNP and oligomycin also stimulated FABP4 secretion. Treatment of adipocytes with insulin during hypoxia reduces FABP4 secretion. Removal of glucose from media and substitution with pyruvate reverses the inhibition of secretion during hypoxia by insulin, indicating that the primary role of FABP4 secretion is to alleviate energy crisis by increasing glucose uptake into fat through increased insulin secretion.
Conclusion: Taken together, these data are indicative of an adipo-insular axis mediated by FABP4, which functions to coordinate insulin secretion and glucose uptake in response to the prevailing needs of adipose tissue.

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Interleukin-6 as a key mediator of metabolic adaptation to fasting
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Background and aims: Fasting leads to increased production of ketone bodies from non-esterified fatty acids (NEFA) liberated from white adipose tissue. However, it is not known whether interleukin-6 (IL-6) contributes to this fasting-induced lipolysis. Thus, the aim of the present study was to analyze whether IL-6 contributes to the metabolic switch from carbohydrate to fat oxidation (metabolic flexibility) provoked by food deprivation (fasting) in lean mice, and if yes, whether this metabolic switch is maintained in obesity and insulin resistance.
Materials and methods: Experiments were performed in C57BL/6J mice fed regular chow or high fat diet (HFD) for 8 weeks and in IL-6 KO mice. Mice were fed ad libitum or fasted for 24 hours and blood was sampled after 0, 6, 12 and 24 hours. Body weight as well as blood glucose and ketone levels were determined. In addition, plasma IL-6, leptin, insulin and NEFA levels were measured and IL-6 mRNA levels were analysed.
Results: The transcription of IL-6 was significantly induced in skeletal muscle but not in white adipose tissue in chow-fed C57BL/6J mice after 6 hours of food withdrawal. Concomitantly, circulating IL-6 levels increased by 3-fold (p<0.05) without changes in plasma insulin levels. Moreover, increased circulating IL-6 levels upon 6 hours of fasting were accompanied by elevated ketone levels (0.50±0.04 mmol/l in fasted vs 0.25±0.04 mmol/l fed mice; p<0.01), an effect that was blunted in IL-6 KO mice (0.48±0.09 mmol/l in WT vs 0.24±0.01 mmol/l in IL-6 KO mice; p<0.05) and chow-fed mice injected with neutralizing IL-6 antibody (0.65±0.03 mmol/l in IgG vs 0.38±0.05 mmol/l in IL-6 nAB injected mice; p<0.001). In addition, HFD-fed mice showed no increased circulating IL-6 levels upon fasting, paralleled by a blunted increase in ketone bodies in the first 6 hours of food withdrawal compared to chow-fed mice (0.37±0.04 mmol/l in chow-fed vs 0.15±0.04 mmol/l in HFD-fed mice; p<0.01).
Conclusion: IL-6 contributes to fasting-induced lipolysis and metabolic flexibility.
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New adipokines identified as downstream targets for adiponectin: lessons from adiponectin-overexpressing or deficient-mice
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Background and aims: Adipokines play a central role in the pathogenesis of the metabolic syndrome. Adiponectin (ApN) is a master regulator of immune

and fuel homeostasis. Identifying downstream adipokines targeted by ApN may help in understanding its action. We have generated transgenic mice allowing persistent and moderate overexpression of ApN (ApN-Overex) specifically in white adipose tissue (AT)(by using the aP2 promoter). We took advantage of this model to unravel the adipokine secretion profile triggered by ApN in AT. We also examine whether a reverse profile occurred in ApN-knockout (ApN-KO) mice.
Materials and methods: To investigate the early and specific effects of ApN, mice were studied at 10 wks of age (before any changes in adiposity or in circulating glucose/lipids). AT was fractionated into adipocytes and stromal-vascular cells (SVC), which were cultured for 8 h. Medium was screened by cytokine antibody arrays allowing the detection of 144 cytokines. Secretion of relevant adipokines was quantified by ELISA and gene expression by RTQ-PCR. NF-κB activity was measured by ELISA and Jun N-terminal kinase (JNK) phosphorylation by western blot.
Results: Profiling of secretory products by antibody arrays showed that ~10 cytokines from each cellular fraction roughly differed between ApN-Overex mice and wild type (WT) mice. These adipokines were quantified by ELISA. When compared to WT mice, the secretion of 3 pro-inflammatory factors (IL-17B, IL-21, TNF-α) and 3 hematopoietic growth factors (GF)(Thrombopoietin, TPO; Granulocyte-, GCSF and granulocyte macrophage-stimulating GF, GMCSF) was reduced in adipocytes of ApN-Overex mice. In SVC of these mice, besides the hematopoietic GFs, the secretion of another GF (vascular endothelial GF receptor 1, VEGFR1), 2 chemokines (RANTES, ICAM-1) and 2 pro-inflammatory factors (IL-6, IL-12p70) was reduced as well. Only one cytokine was oversecreted by SVC of ApN-Overex mice: interleukin-1 receptor 4 (IL-1R4) that exhibits anti-inflammatory properties. Most of these changes in secretion were due to corresponding changes in mRNAs. To investigate whether these changes were specifically induced by ApN, we searched for a reverse profile of adipokine expression in mice lacking ApN. TPO gene expression was increased in adipocytes of ApN-KO mice, and the expression of VEGFR1, IL-12p70 and ICAM-1 was augmented in SVC of these mice. Concomitantly, IL-1R4 expression was reduced in SVC of ApN-KO mice. We next investigated the molecular pathways underlying these inflammatory changes. NF-κB activity was remarkably reduced in AT of ApN-Overex mice, while JNK phosphorylation was unaffected.
Conclusion: ApN regulates in vivo the secretion of downstream adipokines, thereby inducing a shift of the immune balance in both adipocytes and SVC toward a less inflammatory phenotype. These downstream adipokines may be new therapeutic targets for the management of the metabolic syndrome.

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Bradykinin 1 receptor inhibition improves glucose tolerance and reduces adipose tissue inflammation in high-fat diet fed mice

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Background and aims: Kinins are proinflammatory peptides which are involved in the control of blood pressure, inflammation and pain. Recently, it was demonstrated that bradykinin 1 receptor (B1R) deficient mice had reduced fasting plasma glucose and insulin levels and were protected against high-fat diet (HFD) induced obesity. It is however not shown whether pharmacological inhibition of B1R affects glucose tolerance and insulin sensitivity. The aim of this study was to establish whether B1R antagonism could improve glycemia and glucose tolerance in obese, insulin resistant mice. Furthermore, the effect of B1R antagonism on inflammatory markers in adipose tissue was investigated.
Materials and methods: Female C57BL/6J mice were fed a HFD (60%fat) for up to 20 weeks. Thereafter, the mice were administered subcutaneously, twice daily for 12 days with a B1R antagonist (SSR240612, Sanofi-Avensis R&D, 10mg/kg/day). Control mice received vehicle only (5% mannitol). Body weight and food intake were registered every second day. After 7 days treatment, glucose tolerance was estimated in an oral glucose tolerance test (OGTT; 2 g/kg). At termination, adipose tissue samples were collected and the expression of inflammatory markers was analyzed using real time PCR.
Results: After feeding the mice with HFD, basal glucose was elevated (9.1±0.1 vs. 7.8±0.1 mM, $p<0.01$) as well as insulin (1.8±0.3 vs. 0.37±0.06 ng/ml, $p<0.001$) compared to lean, normal diet (ND) fed mice, demonstrating significantly impaired insulin sensitivity in the HFD fed mice. One week treatment of HFD fed mice with the B1R antagonist restored basal glucose levels to 7.9±0.2 mM ($p<0.01$) and reduced insulin levels to 0.91±0.1 ng/ml ($p<0.001$) compared to HFD fed control mice, suggesting improved insulin sensitivity in the B1R antagonist treated mice. Body weight was significantly reduced during B1R antagonism ($p<0.001$), where treated mice lost 11% of their body weight compared to HFD fed control mice during the 12 days treatment period. Food intake was slightly reduced during the second week of treatment, although the body weight loss was apparent earlier than any effects on food intake was observed, suggesting other mechanisms than reduced food intake to be responsible for the weight loss. The OGTT demonstrated improved glucose tolerance with reduced glucose and insulin excursions after B1R inhibition. In the HFD fed mice the expression levels of several markers of inflammation were increased in perigaonadal adipose tissue compared to ND fed mice. B1R antagonism resulted in reduced mRNA expression of MCP-1, TNFα, IL-6 and IL-1β in adipose tissue.
Conclusion: B1R antagonism in obese, insulin resistant mice resulted in improved glycemia, reduced basal insulin levels and improved glucose tolerance. These findings, in association with reduced expression of adipocytokines, demonstrate that inhibition of B1R may be a novel treatment strategy for type 2 diabetes.

OP 22 Making and replacing islet beta cells

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Effective revascularisation and enhancement of islet engraftment by cotransplantation of islets with endothelial progenitor cells

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Background and aims: Islet transplantation is an emerging therapeutic option for type 1 diabetic patients. However, it has many obstacles, one of which is impaired revascularization in transplanted islets leading to islet loss. In contrast to solid organ transplantation where the organ is nourished by large vessel anastomosis, avascular islets suffer from hypoxia and malnutrition for a long time as a consequence of absence of effective blood supply, which results in continuous islet loss and ultimately, only a short period of effective graft function. Endothelial progenitor cells (EPCs) are well known to induce neovascularization in diverse ischemic tissues. Here we aimed to increase islet engraftment by cotransplantation of islets with EPCs.
Materials and methods: Porcine islets were transplanted beneath the kidney capsule of athymic nude mice with or without human cord blood-derived EPCs (EPC group or islet only group, respectively). The transplanted β-cells, EPCs, and blood vessels from islets or host mouse were evaluated by insulin, UEA-1 lectin, and BS-1 lectin immunostaining, respectively. The islet function was followed for 4 weeks post-transplantation with random blood sugar level.
Results: The EPC group mice reached euglycemia (random blood sugar<200mg/dL) at 17 days after transplantation, whereas islet only group mice did not even though they showed improvement of glycemic control compared to diabetic sham control mice. The EPC group mice showed significant increase of body weight compared to the islet only group mice, while sham control diabetic mice continuously lost their weight, which finally lead to death around 28 days post-transplantation. In addition, compared to sham control group, both the islet only and EPC group showed detectable porcine insulin level with the fact that EPC group showed higher level of insulin at both fasting and at glucose challenge compared to the islet only group. At 28 days after transplantation, the kidney bearing transplanted islets were removed to evaluate whether euglycemia induction was by transplanted porcine islets themselves or by regeneration of remnant pancreatic islets in the host. After 7 days of nephrectomy, blood glucose levels of the EPC group reached approximately 500 mg/dL, suggesting that the transplanted islets contributed mainly to glycemic control. Immunostaining of the transplanted islets 5 weeks post-transplantation demonstrated that the islets from the EPC group were highly revascularized compared to those of the islet only group, which coincided with the higher β-cell mass in the EPC group. We also tracked the vasculature of transplanted islets at 3, 14, and 35 days post-transplantation to see the time course of vessel ingrowth into the transplanted islets. Compared to the islet only group, the EPC group showed much more blood vessel organization and branching at 3 days and a significant increase in the number and length of vessel ingrowth at 14 days post-transplantation. Importantly, this was associated with a time-dependent increase of insulin(+) areas in the transplanted islets and also, proliferating, Ki-67-insulin double-positive, regenerating β-cells, all of which were significantly increased in the EPC group than the islet only group.
Conclusion: Collectively, we concluded that cotransplantation of EPCs with islets induce better islet engraftment by enhancing graft revascularization and survival/regeneration of islets.
Supported by: IRICT

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Pancreatic islets transplanted intraportally into the liver in mice have a substantially lower blood flow than native islets

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Background and aims: Pancreatic islets are in the clinical setting transplanted intraportally into the liver, but with low long-term success rate. Engraftment of pancreatic islets in the liver has been difficult to study, since the islets virtually disappear out of sight and imaging techniques have too poor resolution for their study. However, experimental studies have indicated a low revascularization of islets at the intrahepatic site, and that ingrowing blood vessels are derived solely from the hepatic artery and not the portal vein. The aim of the present study was to establish a model and quantify the blood perfusion of intrahepatically transplanted islets. Islet vascular density and the contribution of donor blood vessels in the islet revascularization process were also determined.
Materials and methods: Pancreatic islets were isolated from transgenic YC-3.0 mice, which express the yellow chameleon protein 3.0 under the regulation of the β-actin and cytomegalovirus promoters. Islets from these mice have previously been shown to express enhanced yellow fluorescent protein, one part of the hybrid YC-3.0 protein, in all cells. The islets were transplanted intraportally selectively into the right liver lobe of recipient nude mice by temporary occlusion of the other tributaries of the portal vein at the time of islet infusion (200 islets). One month later, blood perfusion of the transplanted islets was determined by a fluorescent microsphere technique and compared to the blood perfusion of native YC-3.0 islets. The vascular density and relative contribution of recipient and donor blood vessels in islet revascularization was evaluated one month post-transplantation using islets expressing green fluorescent protein behind the Tie2 promoter (Tie2-GFP) for intraportal transplantation to nude mice.
Results: The blood flow in the YC-3.0 islets transplanted to the liver was 0.20 ± 0.1 ml/min/mg islet (n=10), which was markedly lower than the blood perfusion of native YC-3.0 islets (3.86 ± 0.54 ml/min/mg islet; n=6). The blood flow in the native islets of the transplanted nude mice was 3.54 ± 0.62 ml/min/mg islet (n=10). Vascular density in the intraportally transplanted islets was decreased, and very few donor endothelial cells could be observed incorporated in the new islet vascular system.
Conclusion: The blood perfusion of intrahepatically transplanted islets is less than 10% of that in native islets when investigated one month post-transplantation. Low numbers of donor blood vessels contribute to the revascularization at the intrahepatic site, which may at least partially explain their insufficient vascular engraftment.
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Clinical and experimental pancreatic islet transplantation to striated muscle: Establishment of a vascular system similar to that in native islets

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Background and aims: Curing type 1 diabetes by transplanting pancreatic islets into the liver is associated with poor long-term outcome and graft failure at least partly due to inadequate graft revascularization. The aim of the current study was to evaluate striated muscle as a potential angiogenic site for islet transplantation.
Materials and methods: The current study presents a new experimental model which is found applicable to clinical islet transplantation. Islets were implanted into striated muscle where after intra-islet vascular density and blood flow were visualized with intravital and confocal microscopy in mice, and by magnetic resonance imaging in three auto-transplanted pancreatectomized patients. Mice were rendered neutropenic by repeated injections of Gr-1 antibody and diabetes was induced by alloxan treatment.
Results: Contrary to liver engrafted islets, islets transplanted to mouse muscle were revascularized with vessel densities and blood flow entirely comparable to islets within intact pancreas. Initiation of islet revascularization at the muscular site was dependent on neutrophils, and the function of islets transplanted to muscle was proven by curing diabetic mice. The experimental data were confirmed in auto-transplanted patients where higher plasma volumes were measured in islets engrafted in forearm muscle compared to adjacent muscle tissue through high-resolution magnetic resonance imaging.
Conclusion: This study presents a novel paradigm in islet transplantation whereby recruited neutrophils are crucial for the functionally restored intra-islet blood perfusion following transplantation to striated muscle under experimental and clinical situations.

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Tracking mouse islet isografts and allografts using a novel magnetic resonance contrast agent, chitosan-coated superparamagnetic iron oxide nanoparticles

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Background and aims: Although only 10% of islet recipients maintain insulin independence, 80% of them are C-peptide positive at 5 years after transplantation. To better understand the fate of transplanted islets, a magnetic resonance (MR) imaging technique has been used to detect superparamagnetic iron oxide (SPIO)-labeled islet grafts. In this study, we utilized a novel MR contrast agent, chitosan-coated SPIO (CSPIO) nanoparticles, to monitor mouse islet isografts and allografts.
Materials and methods: Male C57BL/6 mice were used as donors and male inbred C57BL/6 (syngeneic) and Balb/c (allogenic) mice were used as recipients of islet transplantation. Mouse pancreas was digested by collagenase and islets were purified by density gradient. After being incubated with and without CSPIO (10 mg/ml), islets were examined under transmission electron microscope (TEM) and their insulin secretion was measured by static incubation and perifusion studies. Cytotoxicity was evaluated by fluorescein diacetate and propidium iodide staining for NIT-1, βTC and αTC1 cells. Three hundred islets were transplanted under left kidney capsule of each mouse. After transplantation, 3.0 Tesla MR imaging of the recipients was performed. At the end of study, the islet graft was removed for insulin and Prussian blue staining and TEM studies.
Results: At 8 hours after incubation of isolated islets with CSPIO, TEM showed CSPIO particles located in endocytotic vesicles of both α- and β-cells. The islets incubated overnight with and without CSPIO had comparable insulin responses to high glucose challenges. There was no increased death rates in NIT-1, βTC and αTC1 cells with increasing CSPIO iron concentrations up to 80 μg or incubation time up to 72 hours. At week 0, 1, 2, 3, 4, 5, 6, 8 after syngeneic transplantation, the grafts of CSPIO-labeled islets were visualized on MR scans as distinct hypointense spots homogeneously located at the upper pole of left kidney. Using the contralateral kidney as a reference, the MR signal intensity of CSPIO-labeled and control islet grafts was 81.9 ± 14.0% and 103.8 ± 15.4%, respectively (P=3.68297E-05). At 8 weeks after transplantation, the CSPIO-labeled islet graft was positive for insulin and iron staining. Under TEM, there were several electron dense clumps distributed in the cytoplasm of islets with intact ultrastructure. The electron energy-loss spectroscopy further demonstrated these clumps contained elementary iron. At day 3, 10, 17, 24, 31, 38 and 45 after allotransplantation, MR scans showed hypointense spots at the upper pole of left kidney gradually decreased in size. The histology of CSPIO-labeled islet grafts at day 10, 17, 24, 31, 38 and 45 showed insulin- and iron-staining co-localized in the same areas but the graft size decreased with time.
Conclusion: Our results indicate, after syngeneic and allo-transplantation, isolated mouse islets labeled with CSPIO nanoparticles can be effectively and safely imaged by using MR scanning.
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Generation of pancreatic endocrine cells from human adult fibroblast-like limbal stem cells

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Background and aims: Stem cells might provide unlimited supply of transplantable cells for β-cell replacement therapy in diabetes. The human limbus hosts epithelial stem cells - which sustain the continuous renewal of the cornea - and fibroblast-like stem cells (f-LSCs) - with apparent broader plasticity. The aim of this study was to isolate and characterize f-LSCs from human donors and to test their differentiation potential towards the pancreatic endocrine phenotype.
Materials and methods: f-LSCs were isolated from 14 limbal biopsies. f-LSCs were characterized by flow cytometry and qRT-PCR for the expression of pluripotent markers and self-renewal ability. We then developed a 4-step pancreatic differentiation protocol, lasting 14-16 days, by adding in a step-by-step way factors and supplements which are known to direct/support pancreatic differentiation, such as activin A, bFGF, B27, N2, nicotinamide and exendin-4. The expression of endodermal, pancreatic, islet and β-cell markers was assessed during differentiation by immunofluorescence, flow cytometry and western blot analysis. Presence of secretory granules was assessed by confocal and electron microscopy. f-LSC-derived cells were also investigated for the ability to secrete C-peptide in response to multiple secretory stimuli.
Results: FACS analysis of freshly digested limbal specimens showed significant expression of the pluripotent stem cell marker SSEA4 (mean ± SD: 65.2 ± 7.6%). After 24-48 hrs, the single cell suspension formed floating spherical aggregates ('limbospheres'), which eventually attached to the plastic surface, giving rise to highly proliferating f-LSCs. Adherent epithelial cells were also observed but f-LSCs progressively prevailed. Positivity for SSEA4 was higher in cultures obtained by re-plating limbospheres, which were devoid of epithelial cells (mean ± SD: 90.8 ± 9.6%). $SSEA4^+$ f-LSCs co-expressed Oct4, Sox2, c-Kit, TRA 1-60, TRA 1-81, ABCG2, Thy-1 and CD105. f-LSCs were negative for CD34, CD45, HLA-DR and for the limbal epithelial marker ΔNp63. Staining of CFSE-labelled $SSEA4^+$ f-LSCs showed that cells are characterized by asymmetrical division. f-LSCs treated with pancreatic differentiation protocol transitioned through a series of intermediates similar to those occurring during pancreatic development, as showed by sequential detection of endodermal, pancreatic, islet and β-cell markers (Sox17, FOXA2, Ngn3, PDX1, MafA, ISL-1, β2NeuroD, NKX6.1, Pax4, GLUT2 and glucokinase). From stage 3 cells progressively gathered in clusters resembling human islets. qRT-PCR, immunofluorescence and western blot analysis at the end of differentiation confirmed expression of islet hormones (c-peptide/proinsulin, insulin, glucagon, somatostatin, ghrelin and PP). Quantification of endocrine cells by flow cytometry showed 72.1 ± 5.3% positive cells for C-peptide/proinsulin, 10.6 ± 2.4% for glucagon and 8.2 ± 2.6% for somatostatin. Confocal and electron microscopy indicated presence of secretory granules. Differentiated cells also possessed the ability to secrete C-peptide in response to glucose, KCl and Tolbutamide.
Conclusion: f-LSCs might represent a novel source of autologous, transplantable, insulin-producing cells which could be tested for the reversal of diabetes.

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Metabolic control in patients with type 1 diabetes after autologous peripheral stem cell transplantation (apbsct)

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Background and aims: Type 1 diabetes mellitus is caused by autoimmunological process destroying pancreatic ß cells. APBSCT leads to modulation of immunological system (in terms of elimination of aggression against ß cells), what subsequently leads to alleviation of autoagressive process and to insulin independency.
Materials and methods: In 8 patients (4 female, 4 male, age 26.0±5.0) with newly diagnosed type 1 diabetes APBSCT was performed. Treatment consisted of 2-3 plasmaphereses, hematopoietic stem cell mobilization with cyclophosphamide ($2g/m^2$, on day -16) and G-CSF (10ug/kg from day -15), collection of at least 3 x 10^6/ kg CD34+ cells, conditioning with cyclophosphamide (50 mg/kg/day on days -5,-4,-3,-2 prior to transplant) and antithymocyte globulin (0,5mg/kg/day on day -5, 1,0 mg/kg/day on days -4,-3,-2,-1) followed by stem cells infusion.
In all patients FPG, HbA_1c, C-peptide fasting and after mixed meal, continuous glucose monitoring (CGM) and intravenous glucose tolerance test (IVGTT) 6 months after transplantation were performed. Control group comprised 6 patients (2 female, 4 male, age 26.2±3.1) in whom after diagnosis an intensive insulin therapy was initiated. In the control group CGM and IVGTT were not performed.
Results: Parameters of metabolic control were shown in table 1. All patients 6 months after APBSCT were insulin-free. HbA_1c and FPG were comparable to the control group, but higher C-peptide values were noted. Insulin concentrations in IVGTT were as follows: 8.1; 9.2; 9.0; 8.7; 9.2; 9.1; 9.0; 8.9; 8.6; 7.6; 7.3 uIU/l (basal 5.4uIU/l).
Conclusion: APBSCT seems to be a promising method of treatment of newly diagnosed type 1 diabetes.

Table 1

	Insulin dosage (IU/kg)	FPG (mg/dl)	Fasting CP (ng/ml)	CP after mixed meal (ng/nl)	HbA1c (%)
Patients after APBSCT n= 8	0	111.8±23.9	1.25±0.57	2.62±1.04	6.01±0.63
Control group n= 6	0.4±0,13	112±30.2	0.77±0.2	1.87±0.7	6.1±0.9
p	<0.001	NS	NS (0.06)	NS (0.18)	NS

OP 23 Genes and islets

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Ctnnb1 gene expression is associated with impaired beta cell function of type 2 diabetic donors

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Background and aims: Common TCF7L2 genetic variants are associated with increased risk for development of type 2 diabetes (T2DM). Beta-catenin/TCF7L2-dependent Wnt signaling is involved in pancreas development, islet function, and insulin production and secretion. Nonetheless, no study has yet assessed Wnt signaling in human islets of T2DM patients. We have, therefore, examined the expression of Wnt pathway component beta-catenin (CTNNB1) in islets isolated from the pancreases of non-diabetic and T2DM individuals.
Materials and methods: Islets were prepared from the pancreas of 9 non-diabetic (CTRL) (5M/4F; age 60.25±13.7 yrs; 25.68±3.68 kg/m2) and 6 T2DM (2M/4F; 64.4±7.2 yrs; 27.20±2.65 kg/m2) multiorgan donors. Glucose-stimulated insulin release (static incubation), mRNA expression of beta-catenin, TCF7L2, and insulin (Real-time RT-PCR) were determined. In order to establish a cause-effect relationship, CTNNB1 and TCF7L2 gene expression were suppressed by siRNA in Ctrl and TCF7L2 overexpressed the by plasmid DNA in Ctrl islets.
Results: Glucose-stimulated insulin release was impaired in T2DM islets as compared to Ctrl (Stimulation Index: 1.34±0.32 vs. 3.59±1.72; p<0.05). Both CTNNB1 (0.03±0.01 vs. 0.09±0.03, p<0.001), and TCF7L2 mRNA expression (0.42±0.41 vs. 7.35±3.40, p<0.001) were higher in T2DM than in Ctrl islets. The two gene expressions were positively correlated (R2=0.508; p=0.0028) while they were negatively correlated with Stimulation Index (CTNNB1: R2=0.267, p=0.052; TCF7L2: R2=0.338, p=0.0231). CTNNB1 and TCF7L2 siRNA transfection of Ctrl islets transfected resulted in a significant reduction of respective gene expression (-72.1±6.9% and -32.8±6.1%, respectively), whereas the Stimulation Index increased (+43.7±14.1% and +37.2±17.3% for CTNNB1 and TCF7L2 silencing, respectively; both p<0.05 or less vs. Ctrl). Insulin gene expression was marked increased in siRNA CTNNB1 transfected islets (+36.46±13.3%), compared to the controls (p<0.001). Finally, TCF7L2 overexpression in non-diabetic islets (+55.7±16.3%) was associated with a significant increase of CTNNB1 gene expression (+67.5±17.9%, p<0.001).
Conclusion: CTNNB1 and TCF7L2 gene expression are increased in islets from T2DM isolated islets, and the expression is negatively correlated with impaired glucose-mediated insulin response. Moreover, modulation of TCF7L2 expression is associated with simultaneous changes in CTNNB1 expression and glucose-mediated insulin release. Our results support the hypothesis that 1) alterations of Wnt signaling occur in pancreatic islets of T2DM patients, and 2) TCF7L2 effects may be mediated via consensual changes in beta-catenin.

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Risk genotypes, allele-specific expression and methylation status in human islets at the *KCNQ1* type 2 diabetes-susceptibility locus

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Background and aims: Though the number of Type 2 Diabetes (T2D) associated loci has increased rapidly, in relatively few cases have the mutational mechanisms through which they operate been enumerated. The *KCNQ1* locus harbours 2 independent clusters of variants associated with T2D risk and impaired insulin secretion, the SNPs with strongest association in European populations in each peak being rs231362 and rs163184. Whilst both signals are intronic, one maps directly over a non-coding RNA (*KCNQ1OT1)*, whose transcription is regulated by parent-of-origin specific methylation, and which is known to regulate transcription of several local cell-cycle repressors, including *CDKN1C,* itself implicated in islet-specific growth phenotypes. Associated SNPs at both signals confer T2D risk only when maternally inherited, implicating aberrant regulation of imprinted expression as the probable mutational mechanism. The aim of this study was to determine whether, in human islets, risk genotype status at *KCNQ1* has effects on DNA methylation, total gene expression and allele-specific expression consistent with this proposed mutational mechanism.
Materials and methods: DNA and RNA were extracted from 42 human islet samples of European origin. Associated SNPs in both signals (e.g. rs231362, rs163184) were genotyped (TaqMan), and methylation quantified (Bisulfite pyrosequencing). Total expression of *KCNQ1*, *KCNQ1OT1*, *KCNQ1DN*, *CDKN1C*, *PHLDA2*, *SLC22A18* and *SLC22A18AS* was determined by qRT-PCR. Allele-specific gene expression was assessed by qRT-PCR, differentiating alleles via coding variants, or (for *CDKN1C*) fragment size analysis using a 12bp exonic indel (del171APVA).
Results: All genotypes were in accordance with expected (HapMap CEPH) frequencies, and displayed no departure from Hardy-Weinberg equilibrium. We found no effective tagging SNP for del171APVA, its strongest LD relationship being with rs2237901 (r^2=0.59). With the exception of *KCNQ1DN*, all tested genes were quantifiably expressed in human islets. The clearest evidence of monoallelic expression was found for *CDKN1C*: without exception, samples heterozygous for del171APVA at the DNA level appeared homozygous at the cDNA level. However, we found no relationship between risk genotype and gene expression for any of the tested genes (p>0.05 in all cases). Risk genotype was correlated with methylation status at 2 sites located on the borders of *KCNQ1OT1*'s differentially methylated promoter region. At both *CTCF* and putative *ZAC* binding sites, methylation level increased with risk allele number. The effect was small (increases of 5.5% and 7.5% on top of 40% and 36% respectively) but significant (p=0.0004 for each).
Conclusion: We have performed the first assessment of the association between risk genotypes at the *KCNQ1* locus and regional effects on DNA methylation and gene expression (total and allele-specific) in human islets. We have shown monoallelic expression of *CDKN1C* in human islets and evidence that risk genotypes effect DNA methylation, but further work is required to demonstrate that these have consequences for regional gene expression. Our study demonstrates the complexity of translating association signals into mutational mechanisms.
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Rare variants of the glucagon gene (GCG) associate with serum insulin and plasma glucagon release, type 2 diabetes, and measures of obesity

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Background and aims: The glucagon gene (*GCG*) encodes several hormones crucial for energy metabolism: glucagon, oxyntomodulin, glucagon-like-peptide (GLP) -1 and -2. We hypothesized that variants in *GCG* associate with type 2 diabetes (T2D), obesity, and/or related metabolic traits.
Materials and methods: *GCG* was sequenced in 481 whites with adult or early-onset obesity or non-autoimmune diabetes and in 384 randomly chosen Danes. Twenty-nine different variants were identified and variants, which had minor allele frequency (MAF) ≥2% (rs4664447 and rs7581952) or were likely to be functional (Ile158Val and Trp169Ter) were genotyped in a total of 17,167 Danes (8,662 subjects from the ADDITTION study, 6,164 subjects from the INTER99 study along with 1,820 T2D patients and 521 glucose tolerant subjects.
Results: In a population-based study of treatment-naïve subjects we found that homozygous carriers of the rare A-allele of rs4664447, which is predicted to disrupt an essential splice enhancer binding site, had lower levels of fasting plasma glucose (mean±SD: 4.8±1.2 vs 5.5±0.8mmol/l, *P*=0.004), fasting insulin (22±14 vs 42±27 pmol/l, *P*=0.04) and glucose-stimulated serum insulin (159±83 vs 290±183 pmol/l, *P*=0.01), insulinogenic index (15±9 vs 29±19, *P*=0.04), and adult-height (165±10 vs 172±9cm, *P*=0.0009) compared to G-allele carriers. Following a hyperglycemic arginine stimulation test homozygous carriers of this variant had 40-50% decreased basal and stimulated levels of serum insulin, as well as plasma glucagon and fully processed GLP-2 compared to matched controls (*P* = <0.0001-0.0008). In studies of T2D patients and glucose tolerant individuals, the rare Val-allele of Ile158Val was associated with a higher prevalence of T2D, OR 1.83 (1.05-3.6), *P*=0.04. Finally, G-carriers compared with T-carriers of rs7581952, had lower BMI (26.4±5.8 vs 27.5±5.0 kg/m^2, *P*=0.009) and body weight (76±16 vs 82±17kg, *P*=0.004). Trp169Ter did not co-segregate with obesity or diabetes.

Conclusion: In the present biological candidate gene study in whites we demonstrate that *GCG* harbors rare variants, rs4664447, rs7581952 and Ile158Val with relatively higher impact on glucose metabolism, measures of obesity and T2D prevalence, respectively, than the impact of most common variants shown to influence metabolic traits identified through genome-wide association studies.
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Influence of novel genetic loci affecting glucose and insulin levels during OGTT on islet function in man

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Background and aims: Type 2 diabetes (T2D) is characterized by chronically elevated glucose levels. Impaired insulin secretion and action are hallmarks of T2D. The Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) identified 16 loci associated with fasting and postprandial glucose levels involved in glucose-sensing, signaling, insulin processing and cell proliferation. Only few of them, *ADCY5, DGKB, PROX1*, *GCK* and *GCKR* seem to influence risk of T2D. We investigated whether these genetic variants would also influence change in insulin secretion and/or action over time or proinsulin, glucagon, GIP, and adiponectin concentrations.
Materials and methods: In the Botnia prospective study (BPS), 2,444 non-diabetic subjects followed for a period of 8 years with OGTT and insulin measurements were genotyped for common variants in 16 loci (*PROX1*, *GCK*, *GCKR*, *G6PC2*, *ADCY5*, *SLC2A2*, *DGKB*, *GLIS3*, *ADRA2A*, *CRY2*, *MADD*, *FADS1*, *IGF1*, *VPS13C*, *C2CD4B* and *GIPR*). In the Botnia Prevalence Prevention and Prediction study (PPP) (N=5,200) in addition to glucose and insulin, fasting/postprandial glucagon and GIP levels, and adiponectin were measured.
Results: We could replicate previously observed associations of variants in *MTNR1B* (DI_{PPP}, beta=-0.237, $P=6\times10^{-29}$), *GIPR* (CIR_{PPP}, beta=-0.057, P=0.008), *FADS1* (CIR_{PPP}, beta=-0.060, P=0.002), *GCK* (DI_{PPP}, beta=-0.092, P=0.003) influencing decreased insulin secretion and *MTNR1B* (ISI_{PPP}, beta=-0.066, $P=2\times10^{-6}$) in decreased insulin sensitivity. In longitudinal BPS, we observed *CRY2* variant (CIR, beta= 0.008, $P_{interaction}=0.01$) associated with increased insulin secretion and *PROX1* (ISI, beta=-0.005, $P_{interaction}=0.03$) with decreased insulin sensitivity over time. The glucose raising allele of *MADD* variant was associated with increased fasting and 2hr (beta=0.166, $P=2\times10^{-8}$, beta=0.138, $P=3\times10^{-11}$), and *MTNR1B* with elevated 2hr (beta=0.062, P=0.0005) proinsulin levels, whereas *FADS1* with decreased (beta=-0.079, P=0.0006, beta=-0.051, P=0.002) fasting and 2 hr proinsulin levels. The 2hr glucagon levels were increased in *GLIS3* (beta=0.024, P=0.04), while decreased in *DUSP9* (beta=-0.049, P=0.01) variant carriers. Fasting GIP levels were elevated in carriers of *CRY2* (beta=0.078, P=0.03), and 2hr GIP in *PROX1* (beta=0.041, P=0.03). On contrary, fasting GIP was decreased in *GCK* (beta=-0.158, P=0.006), and (fasting and 2hr) in *GIPR* (beta=-0.087, P=0.03 and beta=-0.091, $P=2\times10^{-5}$) variant carriers. *MTNR1B* variant was associated with lower (beta=-0.073, P=0.0002) adiponectin concentrations.
Conclusion: These results demonstrate that genetic variants influencing glucose and/or insulin levels also show effects on other metabolic traits like, proinsulin (*MADD, FADS1*), glucagon (*GLIS3*, *DUSP9*), GIP (*GIPR*, *CRY2*, *GCK*), and adiponectin (*MTNR1B*). Low GIP levels in carriers of a loss-of-function variant in the *GIPR* gene in islets suggest the importance of non-receptor mediated mechanisms in determining GIP levels.
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Extending criteria for genetic testing increases diagnosis of maturity-onset diabetes of the young

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Background and aims: Increasing diagnosis rate of monogenic diabetes is essential to enable patients to benefit from optimal treatment and early diagnosis of family members. Current testing for Maturity Onset Diabetes of the Young (MODY) is largely restricted to individuals whose clinical features match the original descriptions of MODY families. This selection method has good specificity (94%) but low sensitivity (58%) in our dataset. Extended MODY testing criteria were defined to select subjects with atypical features of clinically diagnosed type 1 (T1DM) or type 2 (T2DM) diabetes (recruited from primary and secondary care), who then underwent re-sequencing of the Hepatocyte nuclear factor 1-alpha (*HNF1A*) and 4-alpha (*HNF4A*) genes.
Materials and methods: In those with apparent T1DM (n=247), *HNF1A/4A* re-sequencing was performed in individuals with residual β-cell function ≥3y from diagnosis defined as random or glucagon-stimulated c-peptide ≥0.2nmol/l (n=20). In those with apparent T2DM (n=291), *HNF1A/4A* re-sequencing was performed in those with diabetes diagnosed ≤30y (n=35) or diabetes diagnosed ≤45y with absence of metabolic syndrome (MS-, n= 53). Diagnosis rates were compared to those meeting standard diagnostic criteria for MODY; diabetes diagnosed ≤25y with parental diabetes (n=14).
Results: In the T1DM group, 2 *HNF1A* mutations were found. Both individuals had random c-peptide ≥0.2nmol/l and positive GAD antibody titres. In those with apparent T2DM, 10 *HNF1A* and 2 *HNF4A* mutations were identified. Mutations were found in 22% diagnosed ≤30y and 16% of MS-. Only 43% of the MODY cases found met current diagnostic testing guidelines. Family investigations have identified a further 11 mutation carriers including 2 with previously undiagnosed diabetes. Overall 24% of subjects have changed treatment following molecular testing.
Conclusion: We found a prevalence of transcription factor-MODY of 0.8% in apparent T1DM and 4.1% in apparent T2DM. Widened genetic testing criteria based on simple pathophysiological features more than doubled MODY diagnosis rates. Subjects with β-cell antibodies should not be excluded from testing.
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The influence of carbohydrate content of diet on glycaemia in GCK-MODY patients

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Background and aims: Heterozygous inactivating mutations in GCK gene encoding glucokinase result in maturity-onset diabetes of the young (MODY). Nutritional intervention remains the treatment of choice for this form of diabetes. An optimal diet formula for GCK-MODY patients remains, however, to be established. This clinical experiment was designed to evaluate the effect of diet carbohydrate (CH) quantity on glycaemia level in GCK-MODY patients.
Materials and methods: We exposed 8 GCK mutation carriers (6 with diabetes and 2 with impaired fasting glucose-IFG) to diet rich in CH (60% of daily calorie intake) for two days, then patients were switched to low CH diet (25% of daily calorie intake) for another two days. The caloric content was equal throughout the whole 4-day period. All patients were supposed to avoid high glycemic index products. Glucose levels were evaluated with continuous glucose monitoring (CGMS, MiniMed, USA).
Results: In 6 GCK-MODY patients glucose levels were significantly higher during exposure to diet rich in CH vs. low CH diet: the mean glycemia was 8.5 mmol/l (range 8.2-8.9 mmol/l) vs. 7.26 mmol/l (range 7.0-8.1 mmol/l), the mean time spent above the target level of 140 mg/dL was 41.4% (range 23%-55%) and 27.6% (range 13%-46%) ($p<0.02$ for both comparisons), respectively. In addition, 4 out of 5 patients experienced episodes of postprandial hyperglycemia above 200 mg/dL lasting for at least 15 min (on average 1.7 episodes/patient/day) when on high CH diet with no such episodes when on low CH diet. Interestingly, the carbohydrate content of meals had no major impact on glucose levels among GCK mutation carriers with IFG.
Conclusion: Postprandial hyperglycemia is observed on high CH diet in GCK-MODY patients. Diet with modestly limited carbohydrate intake may be effective in optimizing metabolic control in GCK-MODY. Carbohydrate restriction seems to have no major impact on glycemias in GCK mutation carriers not meeting formal criteria of diagnosis of diabetes.

OP 24 Childhood diabetes: What is new?

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Is the distinction between immunologic type 1A and idiopathic type 1B-diabetes clinically relevant? A real-life study with 5 years of follow-up in 3302 paediatric patients with diabetes onset prior to age 12

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Background and aims: The current diabetes classification distinguishes patients with immunologic type-1A and idipathic type-1B. However, few studies so far addressed the relevance of B-cell-autoimmunity at onset for the subsequent course of diabetes under real-life conditions.
Materials and methods: The DPV register was started in 1995 on a nationwide basis: In order to monitor and improve the quality of care, relevant data are documented prospectively at 327 specialized diabetes centers in Germany / Austria. By March 2010, 200722 patients are included in the database (63578 patients type-1). 16921 patients had a pediatric onset of type-1 diabetes prior to age 12, in 4254 of them at least two B-cell-antibodies (ICA, IA2, GAD or IAA at diagnosis) were measured. In 3302 patients, a continuous follow-up from diagnosis for 5 years was available (age at onset: 7.1±3.1 years, 50.4% male). Data were analyzed using non-parametrical comparisons for unadjusted and a hierarchical mixed linear model for adjusted comparisons. Mean daily insulin doses per kg and DCCT-equivalent HbA1c were adjusted for age at onset, gender, BMI and insulin regimen using observed marginal frequencies.
Results: No B-cell-antibody was present in 263 patients, 1 antibody only in 790 (AB1+) and 2 or more positive ABs were present in 2249 patients (AB2+). These groups did not differ by gender, age at onset, initial BMI, rate of DKA or HbA1c at onset, while the reported duration of symptoms was slightly longer in AB- (2.84 weeks) compared to AB1+ (2.39) or AB2+ (2.17 weeks, $p < 0.005$). Concomitant thyroid autoimmunity was present in 24.1 % of AB2+-patients compared to 17.6 % in AB1+ and 16.7 % in AB- patients ($p<0.001$, X^2-test). Based on adjusted means, daily insulin requirement was slightly, but significantly higher in double-antibody-positive compared to antibody-negative patients during the first 3 years of diabetes, but not thereafter (1st year: AB-: 0.52 U/kg versus AB2+ 0.55 U/kg, $p<0.05$). After 5 years of diabetes, insulin requirement was 0.83 U/kg in AB-, 0.85 U/kg in AB1+ and 0.85 U/kg in AB2+ patients (n.s.). Throughout the 5-year period, adjusted HbA1c-values did not differ between the 3 groups: 5th year of diabetes: AB-: 7.6 %, AB1+: 7.5 %, AB2+: 7.5 % (n.s.).
Conclusion: In this large cohort of prospectively followed children with type-1 diabetes, the presence of B-cell-autoimmunity at onset had only a small, clinically irrelevant and transient effect on daily insulin requirement, and no effect on metabolic control achieved. Based on antibody assays currently available in routine care, the presence of B-cell-AB at diagnosis is not predictive for disease severity after 5 years.
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Early introduction of roots in infancy associated with advanced beta cell autoimmunity in young children with HLA-conferred susceptibility to type 1 diabetes

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Background and aims: Early introduction of supplementary foods has been implicated to play a role in the development of β-cell autoimmunity. We set out to study the effects of breastfeeding and age at introduction of supplementary foods on the development of β-cell autoimmunity.
Materials and methods: A prospective birth cohort of 6,069 infants with HLA-DQB1-conferred susceptibility to type 1 diabetes was recruited between 1996-2004. Antibodies against islet cells (ICA), insulin, glutamate dehydroxylase and islet antigen 2 were measured at 3 to 12- month intervals. The families recorded at home the age at introduction of new foods and completed for each visit a structured dietary questionnaire. The endpoint was repeated positivity for ICA plus at least one other antibody and/or clinical type 1 diabetes (n=265).
Results: Early introduction of roots (by the age of 4 months) was related to increased risk of developing positivity for the endpoint [hazard ratio (95% CI) for earliest third 1.75 (1.11-2.75) and for middle third 1.79 (1.22-2.62) compared to last third (>4 months), likelihood ratio test $p= 0.006$], independently of introduction of other foods and of several putative sociodemographic and perinatal confounding factors. Introducing wheat, rye, oats and/or barley cereals (p=0.013) and egg (p=0.031) early was related to an increased risk of the endpoint but only during the first 3 years of life.
Conclusion: Early introduction of roots during infancy is independently associated with increased risk of β-cell autoimmunity among Finnish children with increased genetic susceptibility to type 1 diabetes.
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Effects of physical activity on insulin pump therapy in children and adolescents with type 1 diabetes: A randomised controlled trial

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Background and aims: Few papers have been evaluated the effects of physical activity on insulin pump therapy in children with type 1 diabetes. We evaluated the most effective strategy with insulin pump therapy in children with type 1 diabetes undergoing 2 hours of aerobic-anaerobic exercise.
Materials and methods: We enrolled 15 children and adolescents, aged 10-18 yrs (mean±SD 13.1±2.7 yrs), with type 1 diabetes for 7.2±3.3 yrs, (BMI of 20.05±3.05 m/kg2, insulin requirement 0.85±0.15 U/kg/day, HbA1c 7.66 ± 0.81 %), who were using an insulin pump. Exercise (2 h of anaerobic-alactacid, anaerobic-lactacid and aerobic training prepared and supervised by a qualified trainer) has been maintained at the same level during each session (reliability has been evaluated by means of an arm band), and replicated by each patient for four consecutive days, with a different insulin pump scheme randomly assigned. The four schemes were as follow: the first day the pump has been kept active during exercise; the second day the pump has been suspended during exercise; the third day the pump has been suspended after a 'correction' bolus (the amount of the insulin bolus was equal to the basal insulin the patient would have injected during the 2h-exercise, reduced by 30%); the fourth day was as the third day, plus a temporary basal scheme 20% reduced applied 2 h prior and 4 h after exercise.
Results: Keeping the pump active, glycemic profiles were excellent during exercise, but we observed a significant lowering of blood sugar readings 3 h

after exercise (4/15 patients had had mild hypoglycemia), with a subsequent glycemic increase during the night. The suspension of the pump has shown good glycemic profiles, even if with a significant increase 90 minutes after exercise. The 'correction' bolus determined a significant lowering of glycaemia after 90 minutes from the beginning of exercise. The use of temporary basal scheme showed the highest glycemic variability (table).
Conclusion: We conclude that keeping pump active during exercise seems the best option to properly manage exercise in children with type 1 diabetes, with the recommendation to reduce basal rate by 20% for the 2-4 h after exercise, in order to avoid late-onset hypoglycaemia. However, for those sports that do not allow the use insulin pump, suspending the pump might be a good option, if followed by a +20-30% temporary basal for 2-4 h after exercise.

Glycaemic values during and after exercise according to different insulin pump patterns

	During	During	During	During	After	After	After	Night	Night
	T 0 min	T 60 min	T 90 min	T 120 min	T 60 min	T 120 min	T 180 min	Midnight	3 o'clock
Insulin pump active	117±51	139±64	134±79	130±98	121±75	126±45	98±44	175±101	182±84
Insulin pump suspended	141±83	130±73	133±74	132±70	156±73	164±77	131±69	165±115	169±82
Insulin pump suspended + 'correction' bolus	192±131	133±71	100±39 0.005	108±43 0.031	152±69	185±74	144±103	189±109	156±86
Insulin pump suspended + correction bolus + temporary basal	188±124	103±62 0.043	134±62	251±101	236±117	157±100	155±108	184±149	200±113

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How common is common hypoglycaemia? Frequency of hypoglycaemia in insulin treated children <7years. A one year prospective study of self measured blood glucose
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Background and aims: Hypoglycemia is often regarded as the limiting factor when striving for good glycemic control. ISPAD has defined the HbA1c goal as <7, 5 % in children. Preschool children with insulin treated T1DM are prone to have fluctuating p-glucose and frequent hypoglycemias. The cognitive consequences of hyper- and hypoglycemia during early childhood are under debate. Acute hypoglycemia causes discomfort and interrupts playing and other important activities of the child. Fear of hypoglycemia might affect the parents. The aim of this study was to describe the frequency of hypoglycemia in children < 7 years of age with insulin treated T1DM and the number of nights with hypoglycemia during one year.
Materials and methods: Our hospital serves all patients with Diabetes Mellitus younger than 18 years living in the city of Gothenburg, Sweden and surrounding area. All 36 patients who met the inclusion criteria (age<7 years, T1DM with duration > 3 months, patient at our diabetes unit) were invited to participate in a one year prospective multidimensional study ("DU7"). As a part of this study all SMBG was collected prospectively from autumn 2008 until autumn 2009.The parents of 17 children gave informed consent to participate and 14 of them managed to upload >300 days of p-glucose values from their child's glucometer. Data was collected with the software Diasend. HbA1c was measured with DCA Vantage TM Analyzer, which was calibrated in accordance with Equalis standard, and the values were translated into DCCT standard. HbA1c was measured at the study start and at least four times during the year and the mean value of the year was used for every child. Night was defined as 22-06. Hypoglycemia was defined as p-glucose< 4mmol/l. Severe hypoglycemia was defined as seizures or unconsciousness. 14 children (8 boys) aged 4.8 (1.8-6.9) years with a diabetes duration of 2.4 (0.6-4.7) years participated. 11 were on CSII and 3 on MDI when the study started, one shifted to CSII during the year.
Results: The average mean HbA1c was 7.8 (7.1-8.7) %. 7/14 (50%) had mean HbA1c <7.5 %. The mean p-glucose testing frequency was 2849 (1157-5209) per patient year with mean 7.8 (3.2-14.2) values per day. The mean frequency of hypoglycemia detected by SMBG was 241 (150-385) per patient year. The mean number of nights with detected hypoglycemia was 21 (4-42) per patient year. The mean number of night time SMBG was 452 (73-906) values per patient year. One child reported two and one child reported one severe hypoglycemia (21 events per 100 patient years).
Conclusion: The mean frequency of hypoglycemia was 0.66 events per day (or 4.6 events per week). The children were hypoglycemic 6 (1-12) % of the nights. We need to identify age-specific strategies to improve insulin treatment for preschool children. Further data on how to balance nutrition, insulin and physical activity in order to achieve good glycemic control and thereby preserve health and quality of life in the short and long perspective are needed.
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Suboptimal vitamin D status as a risk factor for CF-related diabetes in the Scandinavian Cystic Fibrosis Nutritional Study
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Background and aims: Cystic fibrosis (CF) is the most common life-shortening autosomal recessive disorder in Caucasians. The two main clinical characteristics of CF are progressive pulmonary disease and pancreatic insufficiency. CF-related diabetes (CFRD) is a major complication of CF. With better medical care and longevity, prevalence of CFRD in adult CF population is increasing and reaches up to 30%. CF patients worldwide are vitamin D insufficient. Recent literature suggests that vitamin D might possess certain antidiabetic properties. We aimed to assess the relationship between vitamin D and CFRD, glucose tolerance and HbA1C using cross-sectional data gathered in the Scandinavian CF Nutritional Study.
Materials and methods: 898 CF patients were included (0.25 - 65 years) from 7 centers in Denmark, Norway and Sweden. Serum 25-hydroxyvitamin D (25(OH)D) and HbA1C were measured, oral glucose tolerance test (OGTT) was carried out and vitamin D intake data were gathered using a seven-day dietary food record. Multiple linear regression analyses were performed for CFRD diagnosis, OGTT result and HbA1C as dependent variables, and serum 25(OH)D, vitamin D insufficiency degree, daily food and supplemented vitamin D sources of intake as independent variables. The model was controlled for country and centre, as well as for known CFRD risk factors: age, gender, genotype, liver dysfunction, long-term oral corticosteroid treatment, lung function and pancreatic insufficient vs. sufficient phenotype.
Results: In the group of all patients included in the study, CFRD diagnosis was positively associated with serum 25(OH)D < 30 nmol/L (N=718; adjusted R^2=10,7%; beta=0,06; p=0,031) and vitamin D insufficiency degree (beta=0,025; p=0,033), and negatively associated with supplemented vitamin D per kg bodyweight (beta=-0,035; p=0,045). HbA1C value was positively associated with 25(OH)D < 30 nmol/L (N=698; adjusted R^2=40,0%; beta=0,207;

p=0,009), 25(OH)D<50 nmol/L (beta= 0,147; p= 0,024) and vitamin D insufficiency degree (beta=0,081; p=0,016). In the subgroup of CF patients without CFRD diagnosis, 25(OH)D<30 nmol/L was a significant determinant of the HbA1C value (beta= 0,15; p=0,035). In non-diabetic CF patients younger than 18 years, 25(OH)D<30 nmol/L determined the HbA1C value even more strongly (beta= 0,24; p=0,006). In non-diabetic CF patients, 18 years old or older, 25(OH)D<30 nmol/L did not determine the HbA1C value. Instead, total vitamin D intake per kg bodyweight was negatively associated with HbA1C in this patient group (beta=-0,95; p=0,045).
Conclusion: Increasing vitamin D intake may have some antidiabetic effect. The study supports the proposed role of vitamin D insufficiency in the pathophysiology of diabetes mellitus and substantiates prospective studies.
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Exenatide lowers postprandial glycaemia and increases satiety without side effects in Prader-Willi syndrome
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Background and aims: Prader-Willi syndrome (PWS) is associated with hyperphagia and obesity, the major burdens in management of this complex disease. Pharmacological interventions have been disappointing so far, and behavioural constraints are still the only effective option today. Exenatide has demonstrated to have beneficial effects on appetite suppression and weight loss, in addition to its glucose lowering effects, but it also has significant side effects. To our knowledge, exenatide has not been tested as a suitable intervention against hyperphagia and obesity in PWS. Therefore, we conducted a single dose pilot study to investigate the safety and effectiveness of exenatide on appetite regulation, glucose homeostasis and appetite hormones in PWS and obese control subjects.
Materials and methods: We recruited 8 subjects with PWS and 11 obese controls (OB) matched for age, sex and body fatness, assessed by DXA. Two standardised meal studies were performed, where subjects received either a single dose of 10 ug exenatide or normal saline injected sc 15 min before meal initiation in a single blinded cross-over design. Glucose, insulin, PYY, GLP-1 and ghrelin were measured for 4 hours postprandially. Appetite and satiety were assessed by visual analogue scale (VAS). Resting energy expenditure (REE) was assessed by indirect calorimetry.
Results: PWS and OB were well matched for central and total body fat mass. Fasting glucose, insulin and degree of insulin resistance (HOMA-IR) were similar in both groups. Exenatide was well tolerated in PWS with no side effects recorded, in contrast to marked side effects observed in OB (bloating 55%, nausea 45%, vomiting 18%). Exenatide significantly increased satiety 120 min after meal initiation (PWS, VAS 1.9±0.4 to 5.3±1.1, p<0.05; OB, VAS 3.5±0.7 to 5.4±0.8, p<0.05), but did not reduce appetite in both groups. Glucose and insulin levels were lowered similarly in both groups. Furthermore, GLP-1 and PYY levels were suppressed to a similar degree. However, ghrelin levels were not affected by exenatide in both groups. Fasting REE was not different between groups when corrected for lean body mass. However, the postprandial increase in REE was only observed in obese but not in PWS subjects (121±31 vs. 0±49 kcal/24h, p=0.048).
Conclusion: This is the first report on the use of exenatide in PWS, demonstrating that it is particularly well tolerated and also similarly effective in increasing satiety and lowering glucose as in simple obesity. Our observation of suppressed insulin and unchanged ghrelin levels challenges previous hypotheses on the cause of hyperghrelinemia in PWS, and it also suggests that delayed gastric emptying might be an important mode of action of exenatide. Longer and larger prospective studies should follow to investigate whether chronic administration of exenatide will lead to decreased food intake and weight loss in PWS.
Supported by: Supporters and families with children with Prader-Willi syndrome, SNSF

OP 25 Diabetes morbidity and mortality

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Significant excess mortality in middle-aged men with diabetes
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Background and aims: Several studies indicate that diabetes confers an increased risk for early death. The aim of this study was to explore all cause mortality, site of death and certainty of day of death in a national cohort of patients with diabetes followed for 15 years from diagnosis and compare with healthy controls.
Materials and methods: Patients aged 15-34 years at diagnosis were registered in the national register Diabetes Incidence Study in Sweden (DISS) during 1992 and 1993 (n=879). A healthy control matched for day of birth and sex was selected for each patient (n=837) at diagnosis of diabetes. Vital status of both patients (n=879) and controls (n=837) was ascertained through 2nd March 2009 by linking records to the Swedish Cause of Death Registry. The follow-up period represented a median of 15.9 years (range 1-17 years) and a total of 27173 person years.
Results: During 15 years of follow-up, 3.3% (29/879; 24 men and 5 women) of patients and 1.1% (9/837; 7 men and 2 women) of controls died. The risk for a patient with diabetes to die was almost three-fold increased HR=2.9; 95% CI 1.4-6.2. This risk was confined to men HR=2.8; 95% CI 1.2-6.5. Diabetes was the dominating cause of death among patients, identified as the underlying cause of death in 34% (10/29), and as a contributory cause of death in an additional five cases. The second most common cause of death in patients was circulatory diseases in 17% (5/29). Most patients 55% (16/29) died at home, remaining patients in hospital 28% (8/29) or elsewhere 17% (5/29) compared to controls of whom 33% (3/9; p=0.45) died at home, 33% (3/9; p=1.0) in hospital and 33% (3/9; p=36) elsewhere. Only 55% (16/29) of patients had a specified day of death on death certificates compared to 100% (9/9; p=0.016) of controls.
Conclusion: Adult men with diabetes had an almost three-fold increased risk to die within the first 15 years after onset of diabetes compared with healthy men. Most middle aged patients with diabetes died at home and often without a specified date of death recorded. The care of young and middle aged people with diabetes should consider the life situation.
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Association of tight glycaemic control with nine-year mortality in type 2 diabetes patients
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Background and aims: Results of some clinical trials have recently suggested that intensive glycaemic control in type 2 diabetes patients, even if reducing the risk of cardiovascular events, does not show a benefit in terms of mortality. The aim of this analysis was to assess the pattern of the relationship between glycaemic control and mortality in type 2 diabetes patients, based on observational data.
Materials and methods: The study population consisted of 3665 type 2 diabetes patients (1060 men and 2605 women) participating in the Latvian diabetes survey in 2000. All deaths (n=1429) that occurred within a subsequent nine-year period and causes of death were identified through the Latvian Diabetes Register and the Causes of Death Data Base of Latvia. The Cox proportional hazard model was used to test associations between the baseline HbA_{1c}, broken down in quintiles (Q1-Q5), and mortality after adjusting for sex, age, diabetes duration, and, subsequently, for frequency of blood glucose testing and education.
Results: Lower HbA_{1c} was associated with a lower risk of death in patients without insulin therapy, e.g. the lowest HbA_{1c} quintile (HbA_{1c}<6.76%), compared to the highest (HbA_{1c}>10.14%), was associated with a 40% reduction

of both all-cause (HR 0.57 (95%CI 0.47-0.70)) and cardiovascular (HR 0.57 (95%CI 0.44-0.74)) mortality, adjusted for sex, age and duration of diabetes. However, among insulin treated patients the lowest risk of death was for the second HbA_{1c} quintile (HR for death from any cause was 0.58 (95%CI 0.42-0.81) and from cardiovascular disease - 0.59 (95%CI 0.38-0.90)), but not for the first quintile (corresponding HRs were 0.80 (95%CI 0.56-1.12) and 0.88 (95%CI 0.57-1.35)). Adding the frequency of blood glucose testing and education into the model, albeit both of them were inversely associated with mortality, did not affect the above mentioned relationship (Table). The observed associations did not change substantially after the deaths, which occurred within the first three years, were excluded from analysis: e.g., among insulin treated patients HR for death from any cause was 0.95 (95%CI 0.63-1.42) for the first quintile of HbA_{1c} and 0.55 (95%CI 0.36-0.83) for the second, but HR for death from cardiovascular disease was 0.99 (95%CI 0.59-1.66) for the first and 0.53 (95%CI 0.30-0.91) for the second quintile of HbA_{1c}, compared to the highest quintile.

Conclusion: In insulin treated type 2 diabetes patients moderate (HbA_{1c} between 6.76 and 7.73%), but not tight glycaemic control (HbA_{1c}<6.76%), was associated with better long-term survival. However, tight glycaemic control predicted better survival in patients not treated with insulin.

Association between baseline HbA1c and mortality in 3665 type 2 diabetes patients, 2000 to 2009

HbA1c(%)	All-cause mortality (1429 cases)		Cardiovascular mortality (881 case)	
	Insulin therapy:		Insulin therapy:	
	Yes	No	Yes	No
quintiles	HR* (95%CI)	HR* (95%CI)	HR* (95%CI)	HR* (95%CI)
Q1	0.84 (0.59-1.19)	0.58 (0.47-0.71)	0.90 (0.58-1.40)	0.57 (0.43-0.74)
Q2	0.64 (0.46-0.89)	0.64 (0.52-0.79)	0.64 (0.42-0.98)	0.59 (0.45-0.77)
Q3	0.73 (0.56-0.93)	0.61 (0.49-0.76)	0.74 (0.53-1.03)	0.66 (0.51-0.87)
Q4	0.72 (0.56-0.92)	0.80 (0.65-0.98)	0.85 (0.62-1.15)	0.78 (0.59-1.02)
Q5	1 (referent)	1 (referent)	1 (referent)	1 (referent)

* adjusted for age, sex, duration of diabetes, frequency of blood glucose testing and education

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Diabetes and insulin duration and cancer incidence: a register linkage study in Denmark

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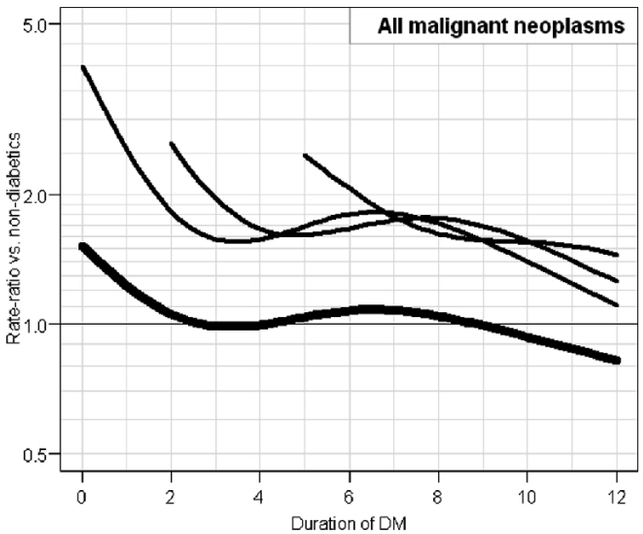
Background and aims: Studies of cancer occurrence among diabetes patients in the past decades have shown elevated rates of cancer of the liver, kidney, female breast and corpus uteri in studies based on up to 8800 cancer cases or 30,000 deaths. Our aim was to extend these studies to assess the effect of diabetes duration and duration of insulin treatment on cancer incidence in the entire Danish population.

Material and methods: We linked the Danish National Diabetes Register and the Danish Cancer Register and followed diabetes patients for the occurrence of cancer and compared with the cancer occurrence in the non-diabetic part of the population. For those diagnosed with diabetes after 1995 we have reliable data on DM duration and the time since first insulin use. For these patients we modelled the effect of DM duration and duration of insulin use on the cancer occurrence rates. We used Poisson models for data in 1-year intervals by age and date of follow-up, date of birth, and in 6-month intervals by duration of disease and insulin treatment.

Results: We had 1.9 mio. years of follow-up, and observed a total of 30,000 cancer cases among the diabetes patients. We found a rate-ratio (RR) of 1.15 for all cancers combined. RRs over 1 were seen for cancers of the digestive tract, with a tendency of decreasing RR from oesophagus to rectum. The RR for liver cancer was elevated (M: 3.7, F:1.8) as well as for pancreas cancers (M and F: 3.0). Other cancer types with a substantially elevated RR were corpus uteri, kidney and lymphomas. Significant but small RRs were seen for lung cancer and female breast cancer. Testis cancer had a RR of 0.8, non-significant. For all cancer types combined, we found the effect of insulin use was highest just after start of treatment, starting at an RR of 2 and decreasing to a stable level of 1.5 after 3 to 4 years after first insulin prescription (based on analyses restricted to DM diagnoses after 1995; 1.1 mio. PY, 18,000 cancers). This pattern was also seen for duration of diabetes, with the risk being highest in the period just after diagnosis at RR 1.5 decreasing to 1 (no excess risk) after 3 years.

Conclusion: Besides the well known elevated cancer risk among diabetes patients, we found that patients on insulin carry an extra risk of cancer, in the order of magnitude of 50% relative to the general population. The effect of diabetes duration and insulin is highest immediately after disease/treatment onset, indicating that it may not be duration or insulin per se that carries the risk, but partly causes common to cancer and diabetes/insulin treatment, such as obesity. This study is the largest of diabetes and cancer incidence so far, and the only one to model the duration effects of both diabetes and insulin treatment. We have however no detailed phenotypic information on the entire Danish population allowing us to control for obesity and other known risk factors for diabetes.

The thick line shows the RR for patients not on insulin, and the thin lines the RR for patients starting insulin 0, 2 and 5 years after disease onset.



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Diabetes and pancreatitis: A population based study to determine the prevalence and incidence of pancreatitis in people with and without diabetes

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Background and aims: Acute Pancreatitis is one of the most common gastroenterological diseases. Incidence of acute pancreatitis has been increasing in the past 40 years. The most common causes of acute pancreatitis are gallstones and alcohol followed by idiopathic aetiology. There has been recent interest in concomitant increase of acute pancreatitis, type 2 diabetes (T2DM), and obesity with their associative risk factors for pancreatitis. The increasing use of incretin based therapies and their possible association with pancreatitis has also added to the debate. With limited published epidemiological data on pancreatitis in people with diabetes, we used the UK General Practice Research Database to investigate if there is an association between diabetes and pancreatitis.

Materials and methods: We identified all patients, ≥ 18 years, who were alive on 1st January 2004 and had at least one year previous history in the database. From these patients a cohort with a diagnosis of T2DM prior to index date was identified. The remaining patients formed the comparator cohort. From both cohorts those with a diagnosis of pancreatitis prior to index date were excluded. The two cohorts were followed forward from index date until the patients' last date to determine the incidence rate of pancreatitis. Relative risk of acute pancreatitis, comparing the two cohorts, was estimated after adjusting for gender and age using Poisson regression.

Results: Of 2.34 million patients aged 18 and over in the database, 75322 (3.2%) had a history of T2DM. Overall 574 people with diabetes (0.76%) had a previous history of pancreatitis (vs. 0.17% in those without diabetes). After

adjusting for age and gender, odds ratio for history of pancreatitis in people with diabetes compared to those without was 3.05 (95%CI: 2.79-3.35). In the incident cohort, we included 74748 people with diabetes and 2,263,766 controls with a mean age of 66 and 48 respectively. The mean follow up was 3.1 years for people with T2DM and 3.2 years for control group. There were 134 incident cases of acute pancreatitis in the people with diabetes and 1975 in the controls. The crude incident rate was 57.5 and 27.4 per 100,000 person years respectively, equal to a ratio of 2.09. After adjusting for age and gender, the relative risk of acute pancreatitis associated with diabetes was 1.47 (95%CI: 1.23-1.76). The relative incidence rate of pancreatitis in different age and sex groups are shown in table.

Conclusion: There is both an increased prevalence and incidence of pancreatitis in people with diabetes compared to those without diabetes. Incidence of pancreatitis in UK general population has increased compared to previous report of 10/100000 per year. The increasing incidence of T2DM might be a contributory factor in increasing incidence of pancreatitis.

Table. Relative risk of acute pancreatitis associated with gender, age, and diabetes

Gender	Age in Years	People with T2 Diabetes (N=74810)				People without Diabetes (N=2,825,782)				Relative Incidence rate Diabetes/No Diabetes
		N	cases	Person years	incidence rate	N	cases	Person years	incidence rate	
Females	18-39	1075	4	3431	116.6	601850	243	1238321	19.6	5.94
	40-49	2603	2	8668	23.1	213151	134	701512	19.1	1.20
	50-59	5203	4	17062	23.4	192829	172	638809	26.9	0.86
	60-69	8625	18	27900	64.5	144650	177	477894	37.0	1.74
	70-79	10095	15	31597	47.5	112157	162	362284	44.7	1.06
	80-	6522	16	17774	90.0	89191	153	248257	61.6	1.46
	All ages	34123	59	106431	55.4	1153828	1041	3667076	28.4	1.95
Males	18-39	1077	1	3332	30.1	420244	189	1311322	14.4	2.09
	40-49	3565	8	11578	69.1	220086	186	717250	25.9	2.66
	50-59	8190	20	26681	75.0	194050	166	638239	26.0	2.88
	60-69	11935	17	38336	44.3	138404	154	450689	34.2	1.29
	70-79	11172	24	34422	69.7	90418	144	284501	50.6	1.37
	80-	4686	5	12394	40.3	46736	95	126602	75.0	0.53
	All ages	40625	75	126744	59.1	1109938	934	3528603	26.5	2.23
Females and Males	All ages	74748	134	233175	57.5	2263766	1975	7195680	27.4	2.09

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Risk prediction of cardiovascular disease in type 2 diabetes - a new risk equation from the Swedish NDR

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Background and aims: Risk prediction models obtained in samples from the general population do not perform well in type 2 diabetes (T2DM) patients, and 5-year risk estimates are proposed more accurate than 10-year risk estimates. We assessed the association of risk factors with cardiovascular disease (CVD), in order to elaborate a risk model for the absolute 5-year risk of CVD in patients with T2DM from the Swedish National Diabetes Register (NDR).

Materials and methods: Investigational sample consisted of 20,571 female and male patients aged 18-70 years, 14% with a history of CVD before baseline in 2002-03, with 1,776 fatal/nonfatal CVD events when followed for 5 years from 2003 to 2007. A separate sample of 2,898 female and male patients aged 18-70 years, was used for application of the risk model, 14% with previous CVD, with 223 CVD events when followed for 4 years from 2004 to 2007.

Results: Adjusted hazard ratios at Cox regression for fatal/nonfatal CVD for a 1 standard deviation increase in continuous variables were: 1.55 for T2DM onset age; 1.53 for T2DM duration; 1.19 for Total-/HDL-Cholesterol ratio; 1.13 for HbA1c; 1.12 for systolic BP; 1.07 for BMI; and dichotomous variables, 1.51 for male gender; 1.44 for smoking; 1.29 for microalbuminuria; 1.49 for macroalbuminuria (>200 µg/min); 1.67 for atrial fibrillation and 1.73 for previous CVD. All 12 variables were used to elaborate the risk equation for 5-year CVD risk. Calibration was excellent when assessed by comparing predicted 5-year risk, mean 10.34±7.1%, and observed 5-year failure rate at survival analysis, 9.94 (95% CI 9.48-10.43) %, with a ratio of 1.04. Figure 1 shows the association between predicted and observed survival rate for CVD. Discrimination was sufficient, with a receiver operator curve (ROC) statistic of 0.70 at logistic regression, and with sensitivity for predicted risk ≥5%; 95% and specificity for risk <10%; 63%. Application of the 4-year CVD risk estimated with use of the presented risk model in another 2,898 separate T2DM patients followed for 4 years still showed a good calibration when comparing predicted 4-year risk, mean 8.80±6.2%, and observed 4-year failure rate at survival analysis, 7.81 (95% CI 6.89-8.86) %, with a ratio of 1.13. Discrimination was sufficient, with a ROC-statistic of 0.73, and with sensitivity for predicted risk ≥5%; 96% and specificity for risk <10%; 72%.

Conclusion: This risk model for the 5-year CVD risk based on 12 predictors, elaborated in a large observational study from the normal population of T2DM, showed adequate calibration and discrimination, and should be useful for clinical practice. However, the risk model also needs to be tested in samples including patients with T2DM from other countries or regions.

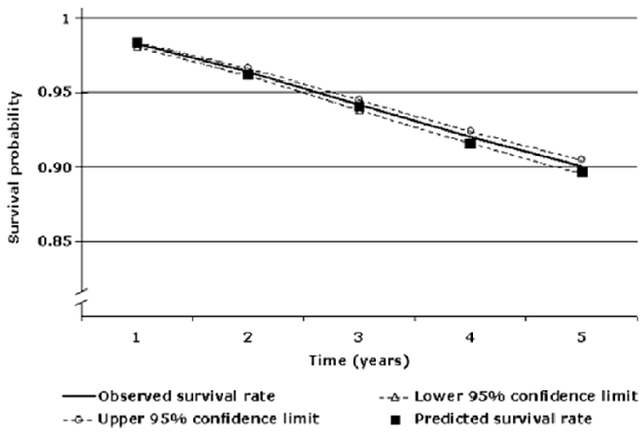

Figure 1

Supported by: The Swedish Association of Local Authorities and Regions funds the NDR

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A new risk model for cardiovascular disease in type 1 diabetes

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Background and aims: Risk prediction models are lacking in patients with type 1 diabetes. We assessed the association of risk factors with cardiovascu-

lar disease (CVD), in order to elaborate a risk model for the absolute 5-year risk of CVD in patients with type 1 diabetes from the Swedish national diabetes register (NDR).
Materials and methods: 4601 female and male patients aged 20-70 years, 5.4% with a history of CVD before baseline in 2002, with 206 fatal/non-fatal CVD events when followed for 5 years from 2003 to 2007. Another sample of 5501 patients aged 20-70 years used for application of the risk model, 5.5% with previous CVD, with 219 CVD events when followed for 4 years from 2004 to 2007.
Results: Adjusted hazard ratios at Cox regression for fatal/nonfatal CVD with 1 standard deviation increase in continuous variables were 3.42 for diabetes duration, 1.62 for onset age, 1.26 for HbA1c, 1.23 for the ratio total-cholesterol : HDL-cholesterol, 1.14 for BMI, and 1.08 for systolic BP, and with the dichotomous variables smoking 1.94, macroalbuminuria (>200 μg/min) 1.49, a history of CVD 2.53, adjusted also for sex. All nine variables were used to elaborate the risk equation for 5-year CVD risk. Calibration was excellent when assessed by comparing predicted 5-year risk, mean 4.4±7.4%, and observed 5-year failure rate at survival analysis, 4.5 (95% CI 3.9-5.1) %, with a ratio of 0.98. Discrimination was sufficient, with a receiver operator curve statistic of 0.87 at logistic regression, and with sensitivity and specificity for the highest quartile of predicted risk (≥5%), 80% and 78% respectively. The presented risk model was applied in a separate sample of 5501 patients followed for 4 years and still showed good calibration when comparing predicted 4-year risk, mean 3.6±6.2%, and observed 4-year failure rate at survival analysis, 4.0 (95% CI 3.5-4.6) %, with a ratio of 0.90. Discrimination was sufficient, with a receiver operator curve statistic of 0.83, and with sensitivity and specificity for the highest tertile of predicted risk 83% and 69%. Figure 1 shows the association between predicted and observed survival rate for CVD.
Conclusion: This risk model for the 5-year CVD risk based on nine predictors, elaborated in a large observational study from the normal adult population of type 1 diabetes, showed adequate calibration and discrimination, and should be useful for clinical practice. However, the risk model also needs to be tested in patients with type 1 diabetes from other countries.

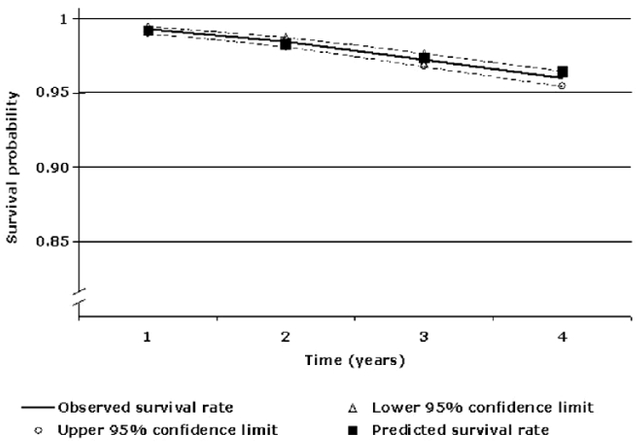

Figure 1. 5501 patients used for application of the risk model.

OP 26 Hypertension and retinopathy

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Risk of hypertension in people with IGT: effect of postprandial glucose control

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Background and aims: Already prediabetes is associated with a high prevalence of hypertension. There exists now evidence from prospective clinical trials that people with impaired glucose tolerance (IGT) represent a high risk group for development of hypertension. So far, little is known on risk factors and impact of glucose control in the prediabetic stage on development of hypertension. This report analyses (1) risk factors for hypertension (2) effect of glucose control by acarbose on incidence of newly diagnosed hypertension in the data pool of the randomized placebo-controlled STOP-NIDDM trial.
Materials and methods: In this multinational trial 14, 742 subjects (age 40-70 years, BMI 25-40 kg/m², > 95 % Caucasians) were screened with a 75 g oGTT, 1,429 eligible patients with IGT were randomised, 1,368 were valid for ITT analysis, mean follow-up time 3.3 years.
Results: At baseline 666 (48.7%) (341 placebo, 325 acarbose) patients were normotensive and 702 (51.3%) had a hypertension (BP>=140/90 mmHg and/or antihypertensive drugs), 96 (14.4%) developed hypertension, annual progression rate of 4.4%. In the intervention group 10.5% developed hypertension vs. 18.2% in the placebo arm. Patients with subsequent development of hypertension had significantly higher levels of blood pressure at baseline. In univariate analysis of time to development of hypertension large waist circumference, metabolic syndrome and treatment group were the only significant predictors. Multivariate analysis confirmed only treatment group as significant variable with a hazard ratio in favour of acarbose of 0.59 (C.I 0.35-0.90).
Conclusion: In about any second subject IGT was associated with hypertension before glucose lowering treatment. At follow-up IGT was accompanied by a high incidence of hypertension (annual rate 4.4%). Control of postprandial hyperglycemia by acarbose reduced significantly rate of newly diagnosed hypertension.

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Cardiorespiratory fitness and reduction in blood pressure and insulin resistance during lifestyle intervention

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Background and aims: Lifestyle intervention, in general, is effective for improving arterial hypertension and other cardiovascular risk factors. However, there is a large variability in these responses. Because high cardiorespiratory fitness (CRF) protects from cardiovascular disease and mortality, we determined whether CRF at baseline predicts the improvement of blood pressure, insulin resistance and other cardiovascular risk factors during a lifestyle intervention.
Materials and methods: A total of 219 subjects at risk for type 2 diabetes, who underwent a 9 months lifestyle intervention with diet modification and increase in physical activity, and had measurement of CRF, were studied. Insulin sensitivity was estimated during a 75g oral glucose tolerance test. Total body-, visceral- and liver fat were measured by magnetic resonance (MR) tomography and ^{1}H-MR spectroscopy. CRF was estimated during incremental cycle exercise (maximal aerobic capacity-VO_{2max}) and motorized treadmill (individual anaerobic threshold-IAT) tests.
Results: During the intervention adiposity, glycemia, CRF and insulin sensitivity largely improved (all $p<0.0003$), however, blood pressure and serum lipids only moderately decreased (all $p<0.06$). High CRF at baseline predicted a larger decrease in systolic ($p<0.0002$) and diastolic ($p<0.004$) blood pressure, and a larger increase in insulin sensitivity ($p<0.04$), but not change in serum lipids (all $p>0.06$).While weight loss was similar among quartiles of CRF ($p>0.17$), systolic ($p<0.0009$) and diastolic ($p<0.01$) blood pressure only decreased in the higher two quartiles. For 1 SD increase in CRF at baseline

the odds ratio for resolution of hypertension or prehypertension was 2.26 (95% CI, 1.40-3.80) for IAT and 1.75 (95% CI, 1.08-2.89) for VO_{2max}.
Conclusion: We provide novel data that measurement of CRF at baseline helps to predict the effectiveness of a lifestyle intervention in improving blood pressure and insulin sensitivity in humans.
Supported by: DFG (KFO 114) / DFG (Heisenberg Stipend to NS)

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Static and dynamic retinal vessel analysis in normo- and hypertensive type 1 diabetic patients
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Background and aims: There is evidence that retinal vessel dilation results in disturbed autoregulation of retinal microcirculation in diabetic retinopathy (DR). On the other hand, stimulation of the retina with flicker light increases retinal vessel diameters in humans. The reduction of flicker light-induced vasodilation is considered endothelial dysfunction. We investigated the static calibre of retinal vessels and retinal vasodilation after stimulation with flickering light in type 1 diabetic patients with and without hypertension.
Materials and methods: Participants consisted of 76 control participants, 58 normotensive type 1 diabetic patients and 57 hypertensive type 1 diabetic patients. DR was classified according to the Early Treatment Diabetic Retinopathy Study criteria (no DR, mild- moderate and severe nonproliferative NPDR). The arteriolar retinal calibre (CRAE, µm) and flicker light-induced retinal vasodilatation (percentage increase over baseline diameter) was measured using Dynamic Vessel Analyzer.
Results: In normotensive diabetic patients, after adjustment for age, sex and glycated hemoglobin, patients without retinopathy (209.7 µm) and with mild NPDR (217.6 µm) had significantly wider arteriolar caliber compared to controls (183.2 µm). Patients with severe NPDR (190.9 µm) had significantly reduced CRAE in comparison to patients without DR or with mild NPDR. After adjustment for age, sex, glycated hemoglobin and CRAE the flicker-induced arteriolar dilation decreased with increasing stages of retinopathy (p-trend <0.014). In diabetic patients with hypertension, after adjustment for age, sex and glycated hemoglobin, patients without retinopathy (202.4 µm) and with mild NPDR (198.6 µm) had significantly wider arteriolar caliber compared to controls. Patients with severe NPDR (181.0 µm) had significantly reduced CRAE in comparison to patients without DR. After adjustment for age, sex, glycated hemoglobin and CRAE the flicker-induced arteriolar dilation decreased with increasing stages of retinopathy (p-trend <0.001). Normotensive patients with mild NPDR had, after adjustment for age, sex, glycated hemoglobin and diabetes duration, wider arteriolar caliber in comparison to hypertensive diabetic patients (p<0.007). Normotensive diabetic patients without retinopathy had wider arteriolar caliber compared to hypertensive patients (p<0.058).
Conclusion: In normotensive type 1 diabetic patients the initial stages of retinopathy are associated with wider arteriolar caliber in comparison to hypertensive patients. The arteriolar vasodilation results in disturbed autoregulation of retinal vessels. The distinct arteriolar vasodilation in normotensive type 1 diabetic patients may contribute to increased vulnerability of retinal microcirculation. Additionally, the flicker-induced arterial vasodilatation decreased significantly with increasing stages of retinopathy independent of CRAE. The decreased flicker-induced dilation of retinal vessels of diabetic patients implies the reduced capacity to autoregulate the blood flow in diabetic retinopathy.

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The role of the vascular endothelial growth factor A in the progression of diabetic retinopathy
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Background and aims: The diabetic retinopathy (DR) remains the main cause leading to the blindness among young people. There are many pathological mechanisms of DR progression. Vascular endothelial growth factor A (VEGF-A) is known to be a factor of the neovascularisation of the retina and development of the neovascular glaucoma (NG). The aim of this study was to investigate the level of VEGF-A in the aqueous humor (AH) in diabetic patients undergone to the cataract and glaucoma surgery, to estimate the grade of DR after the cataract operation and to analyze any correlations between the level of VEGF and DR stage.
Materials and methods: The study included 164 diabetic eyes (110 patients, among them 93 had type 2 diabetes mellitus (DM) and 17 - type 1 DM) and 24 nondiabetic eyes (20 patients) as a control group (CG). Glaucoma group consisted of 15 diabetic patients. All patients were operated due to the cataract or glaucoma; the phacoemulsification of the cataract, extracapsular cataract extraction or Ahmed glaucoma valve implantation were carried out. For assessment of VEGF-A the samples of AH were obtained during operation, were prepared by prompt centrifugation (15,000 g*min) and stored at -80°C. The VEGF-A value was analyzed by ELISA. The patient's examination included standard ophthalmological end endocrinological tests before and after operation. The grade of DR was measured using recommendation of WHO (1999). The grading of DR in diabetic patients was performed in 2 week after operation. The follow up period was from 1 till 24 month. Results of data were expressed as Mediana (95% CI). Relationship between the parameters was analyzed using nonparametric criteria.
Results: The VEGF-A value in patients without diabetes (CG) was 78,85 pg/ml (95% CI, 55,72-120,51). Among diabetic patients the DR grade estimated after cataract operation was the following: 10% eyes didn't have sings of DR, 33% had nonproliferative DR, 39% had preproliferative DR and 18% had proliferative DR. The VEGF-A value in diabetic patients without DR sings was 18,3 pg/ml (95% CI, 10,05-57,65), in patients with nonproliferative DR was 51,12 pg/ml (95% CI, 41,6-88,21), in patients with preproliferative DR was 74,5 pg/ml (95% CI, 66,61-113,03), in patients with proliferative DR was 337,56 pg/ml (95% CI, 234,58-422,79), (p<0,05) (fig.1). The patients with the NG had the VEGF-A value of 1634,01 pg/ml (95% CI, 610,69-2657,33), that was 20 times more than in CG.
Conclusion: Our data confirm the possible role of VEGF-A in the progression of neovascularisation of the retina and development of the NG.

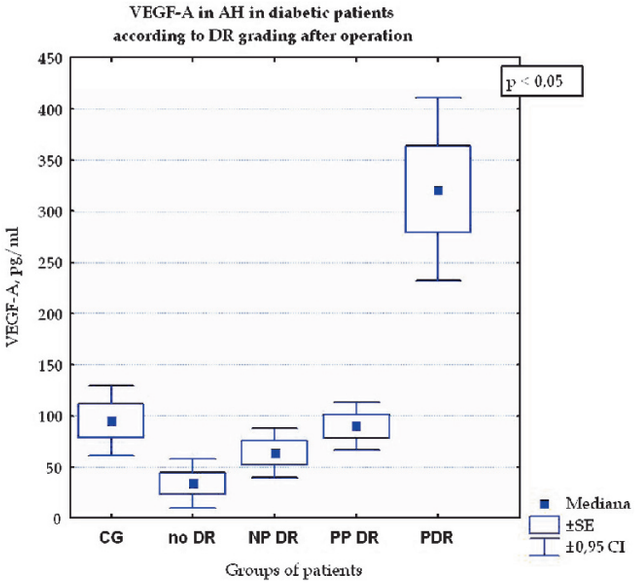

Figure 1: **CG** - control group; **no DR** - diabetic patients without DR; **NP DR** - patients with nonproliferative DR; **PP DR** - patients with preproliferative DR; **PDR** - patients with proliferative DR.

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Immunologic markers at the clinical onset of type 1 diabetes mellitus and the risk of retinopathy 15 years later

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Background and aims: Previous studies have examined the associations of human leukocyte antigen (HLA) genes, islet autoantibodies and residual C-peptide with diabetic retinopathy (DR). In this study we examine the association of these factors, measured at the time of the clinical onset of type 1 diabetes mellitus (T1DM), with DR 15 years later using models to assess the independent effects each of these factors.
Materials and methods: The cohort was first identified in 1992 and 1993 by the Diabetes Incidence Study in Sweden (DISS) which attempts to enroll all incident cases of diabetes for patients between 15 and 34 years of age. Blood samples at diagnosis were analyzed for HLA genotype, islet autoantibodies and serum C-peptide. In 2008, copies of the most recent fundus photographs were obtained from existing patient records. Poisson regression was used to model the relative risk (RR) and 95% confidence interval (95% CI) of DR.
Results: Subjects with HLA DQ6 had a 70% reduced risk of any retinopathy 15 years after the clinical onset of diabetes compared to subjects without any DQ6, DQ8 or DQ2 haplotypes, RR = 0.29, (95% CI: 0.10 - 0.89). In addition, each unit increase in autoantibodies against the 65kD isoform of glutamate decarboxylase (GAD65, GADA) increased the risk of moderate or more severe DR by 45%, compared to subjects with mild or no DR, RR = 1.45 (95% CI: 1.01 - 2.07). C-peptide was not associated with DR.
Conclusion: We have shown that two immunologic factors capable of being determined at the clinical onset of T1DM may be useful to determine the risk of DR 15 years later. Not only does HLA DQ6 provide protection from developing T1DM it may also protect subjects from developing DR. In addition, increased levels of GADA at the time of diabetes onset were associated with the presence of moderate or more severe levels of DR.
Supported by: NHLBI, NIH, KAWF, JF, FVI, LUSCC

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Pericyte-endothelial cell interactions in co-culture models mimicking the physiological and diabetic retinal microenvironment, protective role of thiamine and benfotiamine

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Background and aims: Pericytes regulate vascular tone and perfusion pressure in capillaries, and endothelial cell (EC) proliferation. Their selective loss in the early phases of diabetic retinopathy may cause angiogenesis, due to the failure of their control on endothelium proliferation. We standardized two human retinal pericyte (HRP)/EC co-culture models, to mimick the physiological and diabetic retinal microenvironment. Our aim in this work was to evaluate the interactions between co-cultured HRP/EC in terms of proliferation and apoptosis and the possible protective role of thiamine (T) and its lipophilic analogue benfotiamine (BT) against high glucose-induced damage.
Materials and methods: EC and HRP were co-cultured for 8 days in physiological glucose (NG, 5.6 mmol/l), stable high glucose (HG, 28 mmol/l) and intermittent HG (HGint, 48h HG/48h NG twice), with or without 50 µmol/l T or BT. *No-contact model*: EC were plated on the inner surface of a membrane suspended into the medium and HRP on the bottom of the same well, without physical cell-to-cell contact. *Cell-to-cell contact model*: EC and HRP were plated on the opposite sides of the same membrane, HRP being able to directly contact the abluminal surface of the EC through the pores of the membrane. In control experiments HRP and EC were plated on the relevant surfaces alone. Proliferation (cell counts and DNA synthesis, ELISA) and apoptosis (DNA fragmentation, ELISA) were measured.
Results: In the no-contact model, HG reduced proliferation of co-cultured EC (counts: -23.6%, DNA:-18.7%, $p<0.005$ vs NG), co-cultured HRP (counts: -24.6%, DNA: -12.9%, $p=0.001$ vs NG) and EC alone (counts: -22.5%, DNA: -20.3%, $p<0.05$) and increased apoptosis in co-cultured EC (+21.5%, $p<0.05$ vs NG) and HRP (+45%, $p<0.05$). In the contact model, both HG and HGint reduced co-cultured EC and HRP number (EC: -24.2 and -18.8%, respectively, $p<0.05$; HRP: -29.6 and -38.7%, $p=0.005$) and DNA synthesis (EC:-28.2 and -26.0%, respectively, $p<0.05$; HRP: -25.7 and -26.2%, $p<0.05$), while increasing co-cultured HRP apoptosis (HRP: +75.7 and +150%, $p<0.005$). Stable HG had no effects on HRP in separate cultures. Both EC and HRP proliferated more in co-culture than when cultured alone ($p<0.05$). T and BT countered HG and HGint induced-damage in all cases.
Conclusion: Retinal pericytes may be sensitive to soluble factors, whose nature remains to be clarified, released by the endothelium, cultured in high glucose conditions. Thiamine and benfotiamine are able to counteract this damage, confirming once again their possible role in the prevention/treatment of diabetic microvascular complications.
Supported by: Compagnia di SanPaolo, Regione Piemonte (Torino, Italy)

OP 27 Incretins: mechanistic studies

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Incretins directly suppress the development of macrophage-driven atherosclerosis in apolipoprotein E-null mice

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Background and aims: Several lines of evidence suggest that the incretin-based therapies suppress the development of cardiovascular disease in type 2 diabetes. We investigated the possibility that glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP) can prevent the development of atherosclerosis in apolipoprotein E-null (ApoE$^{-/-}$) mice.
Materials and methods: GLP-1 (1.5 pmol/kg/min) or GIP (17 pmol/kg/min) was continuously infused for 4 weeks into 17-week-old ApoE$^{-/-}$mice fed on atherogenic diet. Alternatively, DPP-4 inhibitor (PKF275-055, Vildagliptin analogue, Novartis) was administrated as drinking water for 4 weeks. Aortic atherosclerosis, oxidized LDL-induced cholesterol ester accumulation (foam cell formation), and its related gene expression in exudate peritoneal macrophages were determined.
Results: Administration of GLP-1, GIP, or DPP-4 inhibitor did not affect food intake, body weight, blood pressure, and plasma levels of lipids, glucose and insulin. Remarkable atherosclerotic lesions in the aorta were observed in 21-week-old ApoE$^{-/-}$ mice. Administration of GLP-1, GIP, or DPP-4 inhibitor significantly reduced the surface areas of atherosclerotic lesions and suppressed atheromatous plaque size and macrophage accumulation in the aortic root as compared with vehicle controls. The suppressive effects of incretins and DPP-4 inhibitor on atherosclerosis were associated with significant decreases in foam cell formation and down-regulation of acyl-CoA:cholesterol acyltransferase 1 (ACAT1) and CD36 in exudate peritoneal macrophages. Incubation with active GLP-1 or GIP but not inactive forms for 48 h resulted in significant suppression of foam cell formation in peritoneal macrophages obtained from non-treated ApoE$^{-/-}$ mice. The suppression of foam cell formation by incretins was totally cancelled by the pretreatment with the receptor antagonists, exendin9-39 or (Pro3)GIP. Both GLP-1 and GIP receptors were detected in the peritoneal macrophages of ApoE$^{-/-}$ mice.
Conclusion: Our study provided the first evidence that both GLP-1 and GIP directly and vildagliptin analogue seen at physiological levels of incretins by DPP-4 inhibition suppress the development of macrophage-driven atherosclerotic lesions associated with down-regulation of essential molecules of foam cell formation such as ACAT-1 and CD36.

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Liraglutide inhibits endothelial cell dysfunction and expression of vascular adhesion molecules in an ApoE mouse model of atherogenesis

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Background and aims: Glucagon-like peptide-1 (GLP-1) is a hormone known to potentiate insulin and lower glucagon secretion. Recently, the once daily GLP-1 analogue, liraglutide, has been approved as a new treatment for type 2 diabetes. Most importantly liraglutide improves glycaemic control and lowers body weight. We have previously shown that liraglutide attenuates TNFα and mild hyperglycemia (10mmol/l)-mediated induction of plasminogen activator inhibitor-1 (PAI-1) and vascular cell adhesion molecule expression in human vascular endothelial cells (hVEC's). Endothelial cell dysfunction (ECD), an early abnormality in atherosclerosis and pre-diabetes, is associated with increased expression of vascular adhesion molecules. The aim of the current study was to determine whether liraglutide was vaso-protective in the non-diabetic ApoE$^{-/-}$ mouse model of ECD and atherosclerosis and establish the dependence of this effect on the GLP-1 receptor (GLP-1R).
Materials and methods: *In vitro studies*: ICAM-1 and VCAM-1 protein levels were determined in conditioned medium from C11-STH hVEC's treated with TNFα (10ng/ml) alone or TNFα + liraglutide (100nM) or TNFα + liraglutide +exendin (9-39)(100nM) for 16hrs using ELISA kits. Semi-quantitative RT-PCR was used to determine mRNA expression of ICAM-1 and VCAM-1. *In vivo studies*: ApoE$^{-/-}$ mice were fed a high fat diet for 16 weeks. Chronic liraglutide treatment was administered in the final 4 weeks via subcutaneous twice daily injection (total of 300μg/kg/day). Treatments included: saline (vehicle; s.c. injection twice daily), liraglutide (s.c. injection of 150μg/kg, twice daily), liraglutide + exendin (9-39) a GLP-1R antagonist (150pmol/kg/min via osmotic mini-pump). Post-treatment the abdominal aorta was divided into four aortic rings per mouse (~3mm long). Concentration response curves to the endothelium-dependent vasodilator acetylcholine (ACh) were constructed in preconstricted aortic rings.
Results: *In vitro* TNFα induced ICAM-1 and VCAM-1 mRNA and protein expression in C11-STH hVEC's at 16 hrs. This effect was significantly inhibited by the addition of liraglutide. The inhibitory effect of liraglutide on TNFα induction of ICAM-1 and VCAM-1 was attenuated by concomitant addition of exendin (9-39). Following these experiments *ex vivo* organ bath studies using isolated aortic rings taken from ApoE$^{-/-}$ mice on a high fat diet treated with liraglutide for 4 weeks demonstrated a significant improvement in endothelial function with liraglutide compared with vehicle treated mice (max relaxation: liraglutide R_{max} = 78.45±2.5% vs 55.36±6.89%; P<0.01; 2-way ANOVA). The improvement in endothelial function was completely abolished in mice co-treated with exendin (9-39) (R_{max} = 53.95±6.54%).
Conclusion: These observations suggest liraglutide significantly inhibits TNFα induction of ICAM-1 and VCAM-1 protein and mRNA expression *in vitro* in a GLP-1R dependent manner. *In vivo* liraglutide treatment significantly inhibited ECD also in a GLP-1R dependent manner. Based on these observations liraglutide may have the potential to attenuate atherogenesis.
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Liraglutide regulates key hypothalamic appetite-related signals in diet-induced obese rats

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Background and aims: Glucagon-Like Peptide-1 (GLP-1) analogues are emerging as an important drug class for the treatment of diabetes and possibly also obesity. While the physiological basis of the anti-diabetic properties of GLP-1 analogues is well understood, less is known about the mechanisms underlying the weight loss effect.
Materials and methods: In the current study we examined the effects of 28 days of daily administration with the GLP-1 analogue liraglutide (0.2 mg/kg) on food intake, body-weight and energy expenditure in male diet-induced obese (DIO) rats compared to vehicle and food-restricted rats (weight-matched to the liraglutide group). Liraglutide reduced food intake (vehicle 530±12 g, liraglutide 439±6.7 g; p<0.0001 vehicle vs liraglutide) and body-weight (vehicle 685±6.0 g, liraglutide 626±5.6 g, p<0.001 vehicle vs liraglutide).
Results: Interestingly, weight-matched animals consumed less food than the liraglutide group (weight-matched 321±0 g; p<0.0001 liraglutide vs weight-matched). In line with these observations, 12-hour energy expenditure measurements at day 14 revealed a near significant increase in oxygen consumption in liraglutide-treated rats (ml/h/kg; vehicle 2051±112, liraglutide 2535±242, weight-matched 2037±200; p=0.12 vehicle vs liraglutide, p=0.089 liraglutide vs weight-matched). Semi-quantitative in situ hybridisations (ISH) on hypothalamic brain sections from liraglutide-treated rats revealed a marked and significant increase in mean cocaine and amphetamine-regulated-transcript (CART) mRNA levels in the arcuate (vehicle 100±15%; liraglutide 161±15%; weight-matched 109±11%; p<0.001 vehicle vs liraglutide) and paraventricular nuclei (vehicle 100±11%, liraglutide 190±30%, weight-matched 116±15%; p<0.01 vehicle vs liraglutide). Arcuate POMC mRNA levels were unchanged (vehicle 100±8.9%, liraglutide 91±0.11%, weight-matched 97±10%), whereas mean NPY (vehicle 100±10%; liraglutide 104±14%, weight-matched 141±10%; p=0.039 vehicle vs weight-matched) and AgRP (vehicle 100±10%, liraglutide 92±14%, weight-matched 174±17%; p<0.001 vehicle vs weight-matched) mRNA levels were significantly elevated in food-restricted rats only.
Conclusion: Our data demonstrate that the GLP-1 analogue liraglutide potently lowers food intake and body-weight possibly by: (1) an increase in arcuate CART mRNA; and (2) by blocking weight-loss-induced increases in arcuate NPY and AgRP mRNA levels.
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Role of lysophosphatidylcholine in GIP secretion by primary K-cells

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Background and aims: Glucose-dependent insulinotropic polypeptide (GIP) is a hormone secreted by enteroendocrine K-cells found in highest density in duodenal and jejunal epithelium. Apart from being a critical regulator of insulin secretion, GIP modulates pancreatic beta-cell proliferation and survival, and controls dietary fat metabolism. GIP has also been postulated to link over nutrition to the development of obesity as pharmacological and genetic interference with GIP signaling proved protective in several rodent obesity models. Although it is known to be secreted in response to the presence of nutrients in the gut lumen, and particularly ingested lipids, the molecular mechanisms involved in the nutrient sensing by K-cells and subsequent secretion of GIP are still unclear. Therefore, understanding these pathways is necessary for the elucidation of the role of the hormone in the development of obesity and type 2 diabetes. The aim of this study was to investigate the effects of lipid micelles on GIP secretion by K-cells.
Materials and methods: GIP secretion was assayed in primary duodenal cultures and STC-1 cells. Ratiometric [Ca2+]i imaging experiments and FRET based [cAMP]i and were performed on STC-1 cells.
Results: Experiments were performed on primary cultures of murine duodenal epithelium and the enteroendocrine model cell line STC-1. To simulate the conditions epithelial cells experience after a lipid rich meal, "postprandial micelles", comprised of oleic acid (200μM), 2-monooleoyl glycerol (70μM), L-α-lysophosphatidylcholine (LPC) (70μM), cholesterol (17μM) and taurocholic acid (TC) (700μM), were applied. Both primary and STC-1 cells responded to lipid micelles by secreting enhanced amounts of GIP (9.2 fold and 3.1 fold stimulation, respectively compared to baseline, $p<0.001$ for both). The stimulation of GIP secretion by lipid micelles was not attributable to cell lysis, as monitored by lactate dehydrogenase activity released into the supernatant. Fluorescence calcium imaging measurements in STC-1 cells, following loading with Fura-2AM, demonstrated elevations in intracellular calcium in response to lipid micelles ($R_{340/380}$ increased 1.8 fold compared to baseline $p<0.001$ n=104). To investigate the relative importance of the different micellar lipids for the secretory response a series of experiments was performed omitting individual components. Exclusion of LPC significantly reduced secretory responses in both primary and STC-1 cells (46% in primary cells n=7 $p<0.05$; 22% in STC-1 n=12, compared to stimulation by micelles containing LPC). Replacement of LPC with phosphatidylcholine (PC) could not compensate (1.14-fold stimulation by micelles containing PC in primary cells; n=4). LPC (in the presence of 700μM TC) promoted the release of GIP in a dose dependent manner over the range of concentrations between 1-100μM. Both in the presence and absence of TC, 70 μM LPC stimulated reversible rises in the cytosolic Ca^{2+} and cAMP concentration monitored in STC-1 cells preloaded with Fura2 or transfected with a Epac2-based FRET-sensor respectively.
Conclusion: Lipid micelles stimulate GIP secretion from primary murine cultures and STC-1 cells. One of the components, LPC, enhanced intracellular concentrations of calcium and augmented levels of intracellular cAMP suggesting the involvement of Gs protein- mediated signaling.
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Exendin-4 inhibits apoptosis of human pancreatic islet endothelial cells in high glucose condition: effects on the AKT/cAMP/PKA signalling pathways

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Introduction: Increased understanding of the glucoregulatory action of incretin hormones has yielded greater insight into the pathophysiology of diabetes and has led to the development of new treatments for type 2 diabetes. Specifically, it has been demonstrated that the endogenous human incretin hormone glucagon-like peptide-1 (GLP-1), the major product from intestinal proglucagon processing, decreases blood glucose by several pathways and that the normal physiologic response to GLP-1 is impaired in type 2 diabetes. Not only does it stimulate beta cell proliferation, but also enhances the differentiation of new beta cells from progenitor cells in the pancreatic duct epithelium and inhibits beta cell apoptosis. The GLP-1 receptor agonists, exendin-4, exhibits actions similar to those of GLP-1, promoting beta-cell growth, survival, insulin secretion and enhancing proinsulin biosyntheses. However, it is established that glucose toxicity is not solely restricted to beta cells, but affects also survival, proliferation and function of pancreatic islet endothelial cells, possibly contributing to beta cell function impairment and beta cell loss. We analyzed the effects of exendin-4 and the pathways involved on cultured human pancreatic islet microendothelial cells (MECs) in hyperglycemic conditions,.
Materials and methods: MECs were cultured in 28 mmol/L glucose concentration up to seven days and, in parallel, stimulated with exendin-4 (10 nM). Apoptosis was evaluated by a photometric enzyme immunoassay measuring mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates as an index of DNA fragmentation, with Hoechst staining of apoptotic cells, and with assay of Caspase 3 activity. Western-blot analysis for P-Akt/Akt, P-Erk/Erk. Bcl-2, Bax were also performed. To evaluate the role of the PI3K/Akt, adenylyl cyclase and PKA pathways, treatments with the inhibitors wortmannin and LY294002, MDL12330A and KT5720 were also performed.
Results: In high glucose condition, proliferation of MECs progressively decreased and apoptosis increased, accompanied by a reduced activation of the survival signaling pathway PI3K/Akt. Incubation with exendin-4 (10 nM) inhibited apoptosis, increasing Akt and Erk phosphorylation and Bcl-2 expression and decreaseing Bax expression. The antiapoptotic effect of the peptides was blocked by inhibition of adenylyl cyclase (AC)/cAMP/protein Kinase A (PKA) and PI3k/Akt signaling pathways.
Conclusion: These results suggest that exendin-4, in addition to its effects on endocrine cells, also promote islet microendothelium survival. The survival effect involves the PI3K/Akt and (AC)/cAMP/protein Kinase A (PKA) signaling pathway. Exendin-4 could therefore represent a potential tool to improve islet vascularization and, indirectly, islet function.
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Chronic treatment with taspoglutide, a once-weekly human GLP-1 analogue, prevents beta cell apoptosis, islet inflammation and fibrosis in ZDF rats

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Background and aims: Our previous data have demonstrated that taspoglutide, a novel once-weekly human glucagon-like peptide (GLP)-1 analog, preserves islet integrity and β-cell function in ZDF rats, a rodent model of insulin resistance and Type 2 diabetes. To further explore the protective effect of taspoglutide on pancreatic islets, its effect on β-cell apoptosis, islet inflammation and fibrosis in vivo was investigated in ZDF rats after chronic treatment.
Materials and methods: Semi-quantitative immunohistochemistry analysis was performed to assess β-cell apoptosis, infiltration of inflammatory cells into the islet core and islet fibrosis on pancreatic sections of 9-week-old male ZDF rats 3 weeks after single application of taspoglutide (1 mg, s.c., formulated to mimic human exposure) or vehicle to 6-week-old ZDF rats (ZDF 6w).
Results: Disease progression in ZDF rats lead to a pronounced decrease in β-cell survival. In particular, the proportion of apoptotic β-cells increased significantly in vehicle-treated rats at the age of 9 weeks when compared to ZDF 6w ($p<0.001$). Conspicuously, the number of apoptotic β-cells correlated with the total number of β-cells per islet cross-section in the 9-week-old diabetic animals. In addition, a significant higher number of apoptotic β-cells was present in the islets located in the pancreatic head (near the duodenum) than in islets located in the pancreatic tail (near the spleen). Taspoglutide-treatment improved β-cell survival as demonstrated by a significantly reduced number of apoptotic β-cells per islet cross-section (taspoglutide: 0.06 vs vehicle: 0.19, $p<0.001$ and n.s. vs. ZDF 6w). This protective effect was even superior in the pancreatic tail. In addition, vehicle-treated ZDF rats at the age of 9 weeks demonstrated a significant increase in infiltration of inflammatory cells into the islet center and the progression of fibrosis within and in close distance to the islets when compared to ZDF 6w and this effect was more pronounced in the pancreatic head. Taspoglutide prevented this inflammatory process as demonstrated by significantly less infiltration of inflammatory cells into the islet center and prevented islet fibrosis. In analogy with the apoptosis results, prevention of fibrosis was more effective in the pancreatic tail.
Conclusion: In summary, these results demonstrate for the first time new aspects of β-cell protective effects of incretins in vivo. In particular, in ZDF rats after chronic treatment protective effects of taspoglutide, a novel once-weekly human GLP-1 analogue, on 1) β-cell apoptosis 2) islet inflammation and 3) islet fibrosis were shown.
Supported by: Roche

OP 28 Targeting of beta cell genes *in vivo*

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Beta cell proliferation is impaired in the absence of survivin in the pancreas of duct-ligated adult mouse

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Background and aims: As type 1 and type 2 diabetes result from absolute or relative deficiencies in beta cell mass, respectively, understanding how beta cell mass is determined and can be manipulated may lead to new therapeutic options. We previously showed that transient perinatal expression of survivin,the inhibitor of apoptosis protein,is essential for pancreatic beta cell mass establishment by regulation of cell cycle progression. This study was designed to determine whether survivin was required for regeneration of beta cell mass in the pancreas of duct-ligated adult mouse.
Materials and methods: Using the Cre-loxP recombination system, we generated a rat insulin promoter (RIP)-driven survivin (also known as Birc5) knockout mouse with a specific deletion of survivin in pancreatic beta cells. Adult RIPCre^{+}survivin$^{flox/flox}$ mice and their control littermates (RIPCre^{+}survivin$^{+/+}$) were subjected to partial pancreatic duct ligation (PDL) or a sham operation, after which islet expression of survivin, beta cell function, beta cell mass, proliferation, beta cell size and apoptosis were analyzed.
Results: In control mice, PDL stimulated beta cell mass regeneration and beta cell proliferation and activated survivin reexpression in beta cells in the ligated tail of pancreas within 2-week. At day 7 post-PDL, control mice underwent significant regeneration of beta cell mass, increase of beta cell proliferation and beta cell numbers in the ligated tail of pancreas. However, targeted deletion of survivin in beta cells exhibited glucose intolerance at day 7 after PDL, with specific impairments in beta cell mass regeneration, beta cell proliferation and pAkt expression, and with larger average beta cell size and nucleus size. Although the number of beta cell clusters was markedly decreased, the mutant mice specifically exhibited an increased proportion of small beta cell clusters (one to ten beta cells) within the ligated tail of pancreas. Additionally, islet architecture, beta cell development and apoptosis were not affected by absence of survivin after PDL.
Conclusion: Our results indicate that survivin reexpression in the pancreatic beta cells after PDL is essential for beta cell mass regeneration through beta cell proliferation. The preexisting beta cells seemingly exhibit a stronger requirement for survivin than new beta cells formed by neogenesis. This study highlights the importance of preexisting beta cell proliferation as a mechanism of beta cell mass regeneration. Beta cell neogenesis, without adequate proliferation, is not sufficient to regenerate a significant amount of beta cell mass after PDL.
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Id1 may play an important role in beta cell dysfunction in type 2 diabetes

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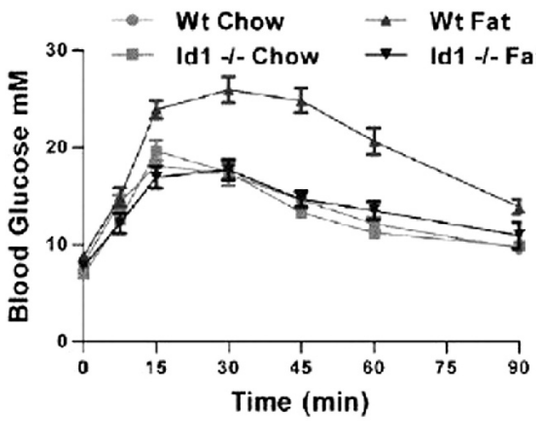
Background and aims: Pancreatic β-cell dysfunction is central to the development of type 2 diabetes (T2D). Chronically elevated glucose and lipid levels may contribute to β-cell dysfunction in T2D, although the molecular mechanisms remain unknown. In islets of diabetic db/db mice, we found that β-cell dysfunction was associated with upregulation of a transcriptional regulator, inhibitor of differentiation 1 (Id1). Id proteins are negative regulators of helix-loop-helix (HLH) transcription factors. HLH proteins are critical for β-cell development and function and therefore we investigated the role of Id1 in insulin secretion and glucose homeostasis in MIN6 cells and Id1 knockout mice.
Materials and methods: Id1 knockout (Id1-/-) and Wildtype (Wt) mice were fed *ad libitum* for 6 or 18 weeks with standard chow (8% calories from fat) or high-fat diet (45% calories from fat [lard]) followed by intraperitoneal glucose tolerance test (*ip*GTT), insulin tolerance test (*ip*ITT) or insulin secretion assay (batch incubations of isolated islets). To examine effects of increased Id1 expression on insulin secretion, Id1 was overexpressed in MIN6 cells followed by insulin secretion assay. Statistical analysis was performed by student's t-test or two-way ANOVA.
Results: Id1-/- mice were completely protected from high-fat diet-induced glucose intolerance (*ip*GTT, $P<0.001$). This was not associated with altered food intake, body weight or epididymal fat pad weight, which were similar in fat-fed Wt and Id1-/- mice. However, insulin levels during the *ip*GTT ($P<0.01$) and insulin release from isolated islets ($P<0.001$) were significantly increased in fat-fed Id1-/- mice compared to fat-fed Wt mice. This protection from diet-induced glucose intolerance in association with augmented insulin secretion was observed at 6 and 18 weeks of high-fat feeding. No differences in insulin action were observed during *ip*ITT of fat-fed Wt and Id1-/- mice, suggesting that the protection from diet-induced glucose intolerance is due to improved β-cell function rather than by changes in insulin sensitivity. No differences in glucose tolerance or insulin secretion were observed in chow-fed Wt and Id1-/- mice, indicating that deletion of Id1 enhances insulin secretion only under conditions of fat oversupply and insulin resistance. In MIN6 cells, overexpression of Id1 led to reduced glucose-stimulated insulin secretion ($P<0.05$), indicating that increased expression of Id1 is sufficient to inhibit insulin secretion.
Conclusion: An important role of Id1 in β-cell dysfunction is supported by evidence that deletion of Id1 in mice protects against diet-induced glucose intolerance and enhances insulin secretion under conditions of fat oversupply, and that increasing Id1 expression in β-cells inhibits insulin secretion. Thus, Id1 expression may contribute to β-cell dysfunction in T2D.



Blood glucose levels during *ip*GTT (2 g/kg) of Wt and Id1 -/- mice fed standard chow or high fat diets. ANOVA: P<0.001 for genotype effect in fat fed mice

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Sodium glucose co-transporter type 2 knockout reduces hyperglycaemia and preserves islet function in db/db mice

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Background and aims: Defective glucose-stimulated insulin secretion by pancreatic beta-cells due to glucose-toxicity has been implicated in the pathogenesis of type-2 diabetes. Therapies that reduce hyperglycemia could therefore not only prevent complications, but could potentially preserve beta-cell function. Inhibition of the sodium-glucose co-transporter type 2 (SGLT2) is a novel insulin-independent approach to lowering plasma glucose. SGLT2 is responsible for reabsorbing the majority of filtered glucose in the kidney. Thus, reducing SGLT2 activity leads to significant loss of glucose in the urine.
Materials and methods: To determine the effects of SGLT2 knockout on glucose homeostasis, insulin action and islet function against a background of extreme insulin resistance (db/db), we characterized wild type (db/db-SGLT2$^{+/+}$), SGLT2 heterozygote (db/db-SGLT2$^{+/-}$) and SGLT2 homozygote (db/db-SGLT2$^{-/-}$) null mice by hyperinsulinemic euglycemic studies as well as with hyperglycemic clamps. Isolated islets from the mice were perifused to assess insulin secretion.

Results: Fasting plasma glucose and insulin were reduced in db/db-SGLT$^{-/-}$ mice relative to db/db-SGLT$^{+/+}$ mice (P<0.001), while there was no difference in db/db-SGLT$^{+/-}$ mice (glucose: 124 ± 8, 199 ± 15, 202 ± 20 mg/dl; insulin: 92 ± 6, 163 ± 22, 176 ± 22 μU/ml). There was no difference in body weight between genotypes, despite a 1.5-3 fold greater daily urine output and glucosuria in db/db-SGLT$^{-/-}$ compared to db/db-SGLT$^{+/+}$ mice. Nevertheless, adiposity was 5% less (P<0.01) in db/db-SGLT$^{-/-}$ mice compared to db/db-SGLT$^{+/+}$. During hyperinsulinemic euglycemic clamp studies, the glucose infusion (GINF) required to maintain euglycemia in db/db-SGLT$^{-/-}$ mice was 100% higher than db/db-SGLT$^{+/-}$ and 170% higher than db/db-SGLT$^{+/+}$ mice (P<0.01). There were no differences in muscle or fat glucose uptake suggesting that reduced hepatic glucose output and/or glucose loss in urine accounted for changes in GINF. Interestingly, *in vivo* islet function as assessed by hyperglycemic clamp studies suggested that SGLT2 inhibition may preserve islet function: AUC insulin secretion was approximately 190% greater in db/db-SGLT$^{-/-}$ mice compared to db/db-SGLT$^{+/+}$ mice (P<0.05). Lastly, perifusion studies with isolated islets demonstrated no difference in glucose stimulated insulin secretion in db/db-SGLT$^{-/-}$ compared to db/db-SGLT$^{+/+}$ mice on a per islet basis, suggesting that changes in beta-cell mass and/or islet number accounted for the increased insulin secretion during hyperglycemic clamps.
Conclusion: SGLT2 knockout reduced adiposity and prevented hyperglycemia in db/db mice. Furthermore, reduced hyperglycemia appeared to preserve overall beta-cell function *in vivo*, resulting in greater insulin secretion during the hyperglycemic clamp. Taken together, these data suggest that SGLT2 inhibition is an attractive therapeutic target for type 2 diabetes.

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Deletion of beta cell Raf-1 kinase results in glucose intolerance
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Background and aims: Regulation of glucose homeostasis by insulin depends on pancreatic beta-cell survival, growth, and function. Raf-1 kinase is a major downstream target of many growth factors that promote growth and survival of many cell types including the pancreatic beta-cells. We have previously reported that insulin protects beta-cells from apoptosis and promotes proliferation by activating Raf-1 signalling in dispersed primary mouse islets and in the mouse-insulinoma MIN6 cell line. As Raf-1 activity also regulates basal apoptosis and insulin secretion in vitro, we hypothesized that Raf-1 may play an important role in beta-cell function in vivo.
Materials and methods: To test this hypothesis, we utilized the Cre-loxP recombination system to obtain a pancreatic beta-cell specific ablation of Raf-1 kinase gene (RIPCre+/-:Raf-1flox/flox) and their littermate controls (RIPCre+/-:Raf-1wt/wt). Immunofluorescense imaging was performed on paraffin-embedded mouse pancreas sections to analyze Raf-1 localization. Quantitative PCR was performed on isolated islet RNA from RIPCre+/-:Raf-1wt/wt and RIPCre+/-:Raf-1flox/flox. Protein expression of Raf-1, B-Raf, ERK1/2, and SNARE proteins were examined by western blotting. Glucose and insulin tolerance tests were performed on RIPCre+/-:Raf-1flox/flox mice and their littermate controls.
Results: Raf-1 is expressed in various cell types in the pancreas including insulin positive cells. Of the Raf isoforms, A-Raf message levels were the highest, followed by B-Raf and Raf-1 in control mice. Beta-cell specific deletion of Raf-1 did not alter the expression of A-Raf or B-Raf message levels in RIPCre+/-:Raf-1flox/flox mice. B-Raf and Erk1/2 protein expression levels did not change in RIPCre+/-:Raf-1flox/flox mice compared to controls. RIPCre+/-:Raf-1flox/flox mice are viable, and no effects on weight gain were observed. RIPCre+/-:Raf-1flox/flox mice have increased fasting basal blood glucose levels, impaired glucose tolerance, but normal insulin tolerance compared to littermates control. Islet perifusion studies demonstrated that islets isolated from RIPCre+/-:Raf-1flox/flox mice have impaired response to both glucose and KCl, consistent with the concept that Raf-1 plays an important role in beta-cell function. To ascertain if Raf-1 function regulates the expression of the exocytotic machinery, SNARE proteins were examined by western blot. In preliminary studies with MIN6 cells, Raf-1 inhibition was associated with an impairment of glucose-stimulated insulin secretion. Furthermore, phosphorylation of synapsin-1, which is required for liberating insulin granules from the actin cytoskeleton, was also completely abrogated.
Conclusion: Together with our previous work demonstrating that Raf-1 promotes survival and proliferation, these new results suggest that Raf-1 positively regulates beta-cell function in vivo.
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Function of Insm1 in mature pancreatic beta cells
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Background and aims: Insm1 was originally isolated from a human insulinoma subtraction library and was subsequently found to be expressed in a large numbers of tumors of neuroendocrine origin as well as in mouse developing nervous systems, many developing and adult endocrine cell types, among them adult pancreatic β-cells. Mouse Insm1 is an intron-less gene, which encodes a protein of 521 amino acids that contains three proline-rich regions, five C2H2 zinc finger motifs, a SNAG motif and a nuclear location signal (NSL). Insm1 is predicted to act as a transcription factor. Our previous work showed that Insm1 is essential for differentiation of pancreatic and intestinal endocrine cells. In the pancreas of Insm1 null mutant mice, endocrine precursors are formed, but only very few insulin-positive β-cells are generated. Instead, endocrine precursor cells accumulate that express none of the pancreatic hormones. However, the function of Insm1 in mature pancreatic β-cells is still unknown
Materials and methods: To define Insm1 function in mature pancreatic β-cells, we introduced a conditional mutation into the Insm1 gene in mature β-cells. For this, we used a floxed Insm1 allele and a tamoxifen-inducible variant of cre, creER, which is expressed under the control of the insulin promoter (RIP-creER).
Results: Conditional mutation of Insm1 in mature pancreatic β-cell blocks glucose induced insulin secretion and causes hyperglycemia. However, Insm1 mutation did not ablate amino acid (e.g. Arginine) stimulated insulin secretion, and the secretory machinery appears therefore not to be defected. Whole pancreatic insulin contains are comparable between wild type and conditional mutant mice, which is consistent with β-cell mass and β-cell number. Microarray analysis shown expression of numerous genes that are important for pancreatic β-cell function is abnormal.
Conclusion: Insm1 is important for maintaining of mature pancreatic β-cell function. Deletion of Insm1 in mature pancreatic β-cells causes diabetic phenotype.

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Deletion of the mitochondrial chaperone prohibitin-2 in beta cells results in beta cell apoptosis and promotes diabetes in transgenic Bet-Phb2$^{-/-}$ mice
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Background and aims: Prohibitins are evolutionary well conserved and ubiquitously expressed proteins having two interdependent homologues, prohibitin-1 and -2 (PHB2). They are implicated in cell cycle regulation, ROS production, cancer, obesity, and inflammation. Prohibitins form ring complexes in the inner mitochondrial membrane, thereby controlling mitochondrial dynamics, cristae remodeling and apoptosis. In pancreatic beta-cells, mitochondria are essential players in the control of metabolism-secretion coupling. Nevertheless, we lack information regarding importance of mitochondrial dynamics in general and putative role of prohibitins in particular. Here, we generated beta-cell specific *Phb-2* knockout mice (Bet-*Phb2*$^{-/-}$) to study prohibitins and mitochondrial integrity in the regulation of glucose homeostasis, islet physiology, and beta-cell mass.
Materials and methods: Bet-*Phb2*$^{-/-}$ mice were generated by crossing mice with *Phb2* allele flanked by lox-P sites (*Phb2*$^{fl/fl}$) with animals carrying the Cre recombinase driven by an insulin promoter (*Rip-Cre*). Mice thus generated (Bet-*Phb2*$^{-/-}$) were assessed by genomic PCR and immunoblotting on isolated tissues. Glucose homeostasis was characterized by glucose tolerance tests and plasma insulin levels. In-situ pancreatic perfusions were carried out to study effects of *Phb2* deletion on glucose-stimulated insulin secretion. Islet morphology, beta-cell mass, proliferation and apoptosis were studied using immuno-histochemistry. The study has been carried out along the principles of laboratory animal care.
Results: Non-fasting blood glucose showed that Bet-*Phb2*$^{-/-}$ mice were normoglycaemic up to the age of 4 weeks and then became hyperglycemic (14.6 mM, p<0.05) at 6 weeks, further developing diabetes with age and eventually causing death at the age 12-15 weeks. Bet-*Phb2*$^{-/-}$ mice grew normally until 6

weeks of age before progressive decline and weight loss. At 6 weeks, ipGTT revealed that Bet-*Phb2*$^{-/-}$ mice were glucose intolerant (AUC +133%, p<0.001) compared with littermate control (*Phb2*$^{fl/fl}$) mice. Plasma insulin levels were lower by 66% (p<0.01) during fasting and 93% (p<0.001) after glucose injection in 6-week old Bet-*Phb2*$^{-/-}$ mice. In-situ pancreatic perfusions revealed that both first and second phases of glucose-stimulated insulin secretion were markedly reduced (-76%, p<0.001 and -78%, p<0.001, respectively) in 6-week old Bet-*Phb2*$^{-/-}$ mice. Up to the age of 4 weeks, beta-cell proliferation was 3 times higher in Bet-*Phb2*$^{-/-}$ versus controls. Bet-*Phb2*$^{-/-}$ islets were disorganized at the age of 4 weeks onwards and beta-cell mass progressively declined with age.

Conclusion: These results demonstrate that prohibitin-2 is essential for beta-cell function and survival. Loss of this mitochondrial chaperone protein led to beta-cell death and diabetes. Interestingly, beta-cell proliferation could compensate apoptosis and postponed diabetes during the first 4 weeks of life of the Bet-*Phb2*$^{-/-}$ mice.

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OP 29 Type 1 diabetes mellitus genetics: expression, interaction and function

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Investigation of the expressional profiles in human pancreatic islets for candidate genes located in 40 type 1 diabetes associated regions

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Background and aims: Today more than 40 non-HLA regions have been demonstrated to be robustly associated with the risk of type 1 diabetes (T1D). However, the causal variants have not yet been identified for any of the genes located within these regions. For most of the genes their function in relation to the disease pathogenesis is also unknown. One way of dissecting possible roles for these genes in the pathogenesis is to investigate their expression, under cytokine stimulation in human pancreatic islets, to mimic the inflammatory process that precedes the clinical onset of T1D. Genes that change their expression can be presumed to have a functional relevance and further investigations can then be focused on these.

Materials and methods: Candidate genes were chosen from the 40 risk loci that were identified in the genome wide association scan published by the Type 1 Diabetes Genetics Consortium (T1DGC). The genes that were located closest to the association signal within each associated region were chosen for evaluation. The gene expression of 47 candidates was evaluated using custom designed Low Density Arrays (Applied Biosystems). Expression levels were measured in eight individual human pancreatic islet preparations before and after cytokine stimulation (mix of TNF-α, IFN-γ and IL-1β). Gene expression levels were normalized against the geometric mean of three different housekeeping genes and compared using the paired t-test.

Results: We detected expression in human pancreatic islets for 30 of the 49 investigated genes, of which 13 were significantly and four borderline regulated by cytokine treatment. Already well known candidate genes, such as *INS* and *IFIH1* were among the significantly regulated genes. Furthermore, among genes located in recently associated regions *IL10* (1q32), *IL7R* (5p13), *MMP19* (12q13) and *TNFAIP3* (6q23) were up-regulated whereas *CTSH* (15q25), *COBL* (7p12), *CTRB2* (16q23) and *SKAP2* (7p15) were down-regulated by cytokines.

Conclusion: Expression profiling of genes located in T1D associated regions has pin-pointed genes that are affected by cytokine stimulation in human islets, thus guiding the investigation towards their functional implication in the pathogenesis. In addition, the results demonstrate that despite that many of the investigated genes have been classified as "immune genes" they are in fact also expressed in human islets, and moreover they are affected by cytokines. Future studies involve the investigation of risk genotype-specific effects on gene expression in human lymphocytes to find out how genetic variation associated with the risk genes can affect their function and lead to a pathogenic state.

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Type 1 diabetes protein network analyses combined with gene expression profiling in human islets

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Background: In type 1 diabetes (T1D) 40 non-HLA regions have been demonstrated to be robustly associated. For none of the regions the causal variant is known and most contain many genes. Even with the genetic contribution from these 40 regions and the HLA region we have still not explained the total genetic heritability for T1D. Novel approaches are needed to identify the causal T1D genes and to explain this missing heritability. We have identified all positional candidate genes in the 40 non-HLA T1D associated regions identified in genome-wide association studies (GWAS) and used these as input for network-based data mining analyses. For all input genes and interaction partners we performed expression profiling in human pancreatic islets.

Methods: 350 positional candidate genes were identified from non-HLA T1D associated LD regions from GWAS using NCBI databases. These genes were used as input into STRING data mining software extracting human protein-protein interaction networks enriched for input proteins. We used custom designed Low Density Arrays (Applied Biosystems) to evaluate gene expression levels of all genes identified as nodes in the networks. Expression levels were measured in eight individual human pancreatic islet preparations +/- cytokine stimulation. Gene expression levels were normalized against the geometric mean of three different housekeeping genes and compared using paired t-test.
Results: We identified 17 interaction networks containing 247 nodes, of which ~40 were input proteins from T1D associated regions. Three networks contained a significant amount of differentially regulated genes upon cytokine stimulation, whereas others contained none or only a few significantly regulated genes. These three networks highlight interesting pathways and shed light on the effect of cytokines on network level The data suggest that both classical inflammatory, but also non-inflammatory, pathways are important disease pathways.
Conclusion: We have used a novel approach to identify networks and genes of importance in T1D. Only few of the significantly regulated genes were located in T1D genetic regions identified in GWAS, so we extracted information not directly obtainable from genetic studies, i.e. GWAS. We believe this approach of combining genetic knowledge with bioinformatics and functional genomics unravels knowledge of relevance for disease pathogenesis from GWAS, which can be used to point at potential novel pathways or targets for new prevention or treatment strategies.

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Identification of type 1 diabetes candidate genes by *in silico* phenome-interactome analysis

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Background: Type 1 diabetes (T1D) has a strong genetic background, but except for a few, the specific genes that contribute to disease remain to be discovered.
Methods: A systems biology/bioinformatics method for predicting genes involved in disease was recently developed. The method is based on *in silico* generation of protein networks and disease phenotype association. We used this "phenome-interactome protein network analysis" to identify novel T1D candidate genes. Follow-up studies involved experiments to address the functional role of predicted genes in pancreatic beta-cells.
Results: Using T1D genome-wide linkage results as input data, we performed a phenome-interactome protein network analysis. The analysis revealed 11 candidate genes including two *HLA* genes and the *INS* gene. A top-scoring candidate gene was Huntingtin-interacting protein (*HIP*)-14. No previous reports have linked *HIP14* to T1D. To explore the potential functional role of HIP14 in pancreatic beta-cells, we performed a series of experiments on different beta-cell model systems. Immuno-histochemical staining and Western blotting indicated that HIP14 is expressed in both primary and clonal beta-cells. Comparison of HIP14 expression in a beta-cell line and an alfa-cell line demonstrated that HIP14 is 2-fold higher expressed in beta-cells versus alfa-cells. Knock-down experiments with either siRNA or short-hairpin RNA against HIP14 in purified primary rat beta-cells or INS-1 cells induced apoptotic cell death suggesting that HIP14 is required for beta-cell survival. Further, knock-down of HIP14 caused a reduction in beta-cell insulin release. Consistent with a pro-survival role of HIP14 in beta-cells, inflammatory cytokines (IL-1 + IFN) known to contribute to beta-cell dysfunction and apoptosis in T1D reduced the expression of HIP14 in rat islets and INS-1 cells. In human islets, cytokines decreased HIP14 expression in 6 out of 8 donor islet preparations.
Conclusion: Using a bioinformatics/systems biology approach to predict disease candidate genes, we, among several other genes, identified HIP14 as a gene being involved in T1D. Functional studies demonstrated that expression of HIP14 is suppressed under inflammatory conditions resembling those of T1D, and that HIP14 is needed for beta-cell survival and insulin secretion.

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Factors associated with early/childhood onset of the disease in families with type 1 diabetes. Results from the type 1 diabetes genetics consortium (T1DGC)

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Background and aims: Families with at least 2 affected siblings have been included by the T1DGC in order to find genes associated with risk/protection for type 1 diabetes (T1D). The aim of our study was to find factors associated with early and childhood onset of the disease.
Materials and methods: Clinical information was obtained with questionnaires, serum anti-GAD and anti-IA2 antibodies were measured (RBA) in the affected siblings and HLA was genotyped (PCR-based, sequence-specific oligonucleotide probe system). Early onset of the disease was defined as onset in the lowest tertile (<6 years), whereas childhood-onset diabetes was defined as that diagnosed before the age of 15. Differences between groups were analysed with Wilcoxon-Mann-Whitney's test and chi-square. The first two siblings per family diagnosed with T1D were included in the analysis. To identify the factors independently associated with early and childhood onset, multivariate regression analysis was performed, including time since diagnosis, antibody positivity, presence of associated autoimmune diseases (AAID) and number of risk and protective HLA haplotypes as independent variables. High-risk and protective haplotypes were defined as the 4 most susceptible and protective, respectively, according to a previous report from the T1DGC.
Results: Data including unequivocal HLA haplotypes was available from 2663 families (4817 participants). Median (range) age of onset of the disease was 9 (0-49) years, time since diagnosis, 7 (0-57) years, 49.3% were female, 47.3% were positive for GADA and 47.2% for IA2A. Subjects with early onset of T1D were less frequently GADA and IA2A positive, had longer disease duration and had more high-risk HLA haplotypes. In the multiple regression analysis, male gender (OR 1.15 (1.02-1.31), p=0.019), negativity to GADA (OR 0.48 (0.42-0.54), p=2*10-16 for positivity) and IA2A (OR 0.68 (0.60-0.78), p=4.25*10-9) and time since diagnosis (non-lineal, p=2.45*10-13) were independently associated with early onset. Participants with childhood onset T1D (84%) were more frequently male, had less frequent AAID, less GADA and more IA2A positivity, less protective and more high-risk HLA haplotypes and longer time since diagnosis than participants with adult-onset T1D. In the multiple regression analysis, the following were independently associated with childhood-onset disease: male gender (OR 1.49 (1.28-1.74), p=2.35*10-7, AAID (OR 0.75 (0.60-0.92), p=0.007), GADA negativity (OR 0.35 (0.30-0.41) for positivity, p=2*10-16) and IA2A positivity (OR 1.30 (1.11-1.52), p=0.001), number of protective HLA haplotypes (OR 0.27 (0.11-0.70) per haplotype, p=0.007) and time since diagnosis (non-linear, p=0.001).
Conclusion: Early onset of T1D was independently associated with male predominance and antibody negativity at examination, whereas childhood onset disease was associated with male predominance, IA2A positivity and GADA negativity, less frequent AAID and fewer protective HLA haplotypes.
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HLA and insulin genes: Bayesian networks confirm interaction between the two most important susceptibility genes in type 1 diabetes in a French caucasian population

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Background and aims: Type 1 diabetes (T1D) is an autoimmune chronic disease resulting from the interaction between more or less favouring environmental factors with multiple susceptibility genes. HLA, Insulin (INS), CTLA4 and PTPN22 are considered the main T1D susceptibility genes. As many epidemiological studies have demonstrated, T1D incidence is increasing worldwide by 3.9% per year, particularly in Caucasian population of

Northern Europe. Unlike in single gene disorders, in multifactorial diseases, such as T1D, identifying the combination of causative genes is still difficult. Genetic profiles of individuals who are affected by T1D appear to change among different countries shifting from mainly high-risk genotypes towards higher percentages of median and low-risk genotypes. In a previous study we demonstrated the interaction between HLA and INS genes in an Italian population from Lazio region using the Bayesian Network approach. To confirm our previous findings, the aim of the present study was to investigate and verify in T1D the dependency and interaction between HLA and INS genes by investigating another Caucasian population.

Materials and methods: We have analyzed a database of genetic data from a French Caucasian population, the case-control cohort consisted of 868 French T1D patients (M/F 1.13, 19.63 ±14.40 yrs mean age of T1D onset) and 93 French control subjects (M/F 0.7). Diagnosis of T1D was based on the ADA classification criteria. We divided HLA alleles in high, moderate and low risk for T1D, PTPN22 alleles in susceptibility/non susceptibility alleles and INS gene alleles in susceptibility/protection. We created a Bayesian Network model trained on genetic variables and group status (T1D/control). Bayesian networks, also called belief networks, are probabilistic graphical models that represent a set of variables and their probabilistic dependencies. To gain insights into the dependency/interaction between susceptibility genes involved in T1D, we have assessed more than one gene at the time (namely HLA, INS and PTPN22 genes).

Results: We implemented a Bayesian Networks model learning the structure of the specified database, with a fixed level of significance equal to 0.05 to find out the interaction. The model showed that group status was directly influenced by HLA (p = 1.0*10^(-26)) and that there was a dependency of INS on HLA (p = 4*10^(-4)). In addition to our previous data, having separated the data group wise, the analysis of T1D patients group also highlighted the gene interaction between HLA and INS (p = 3.7*10^(-4)). No significant relation between HLA and PTPN22 (p = NS) and PTPN22 and "status group" (p = NS) was found.

Conclusion: The presence of interactions between susceptibility genes can explain why the study of a single susceptibility gene in a polygenic disease such as T1D offers limited information. Bayesian network type of analysis represents a step forward in understanding gene interactions and may offer novel clues for T1D pathogenesis. Further studies are needed to clarify the true nature of the biological interaction between HLA and INS gene alleles.

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Functional genomics of the type 1 diabetes gene interferon-induced helicase 1-linked downstream signal mechanisms in humans

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Background and aims: In type 1 diabetes (T1D) beta cell injury develops as a consequence of interaction between a complex polygenic background and environment. More than 40 T1D loci have been identified to date, however, their functional characterization, and their assignment to specific disease endophenotypes is lacking. Recently, a non-synonymous SNP (rs1990760) in the gene encoding interferon induced helicase C containing domain 1 (IFIH1) showed an association with type 1 diabetes. IFIH1 is a cytosolic viral recognition receptor, and may provide a link between viral infections and T1D. In the present study we evaluated the variation in the gene expression profile of IFIH1, and related global gene expression profiles in response to type I interferon and a viral infection model.

Materials and methods: We compared downstream signal mechanisms in T1D children with the wild type AA and the T1D predisposing GG genotypes. Jurkat (T lymphocytic) and MonoMac (monocytic) cells were stimulated with interferon-β or/and polyinosinic acid-polycytidylic acid complex (polyI:C). Global expression profiling was carried out using high density gene expression arrays. Bioinformatics analysis was performed using HT association pattern mining, FDR significance estimation, GO and KEGG pathway annotation.

Results: Treatment of both cell lines with interferon-β resulted in a biphasic induction of IFIH1. The maximum level of IFIH1 mRNA was apparent at 8 h in Jurkat cells and 4 h in MonoMac cells, then decreased before a subsequent increase observed at 24 h postinduction in both cell lines. The expression of IFIH1 mRNA increased in a interferon-β dose-dependent manner. The temporal kinetics of IFIH1 expression after polyI:C induction differed in the two studied cell lines. In a 24 hour time frame, Jurkat cells presented a biphasic induction profile, in the monocytic cells IFIH1 mRNA levels presented a single peak. The combined treatment with IFNβ followed by polyI:C had a distinct effect. Gene network analysis results will be presented on the event.

Conclusion: In the mononuclear cell lines Jurkat and MonoMac, as a result of interferon-β/polyI:C treatment the IFIH1 mRNA level's temporal kinetic presented distinct characteristics in the first phase, but similar in long term effect. The results serve as take-off data for further studies carried out in human peripheral blood mononuclear cells, presenting basis for the analysis of the role of IFIH1 in T1D pathomechanism. The global expression profiling data, and their relation to disease pathways will be released during the presentation.

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OP 30 Insulin action and glucose uptake *in vitro*

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Insulin stimulates human brown adipose tissue glucose uptake effectively

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Background and aims: Brown adipose tissue (BAT) has been acknowledged to be noteworthy in adults but its role in metabolism is still poorly understood. The aim of this study was to investigate the effects of insulin on glucose uptake (GU) and perfusion in BAT depots confirmed by cold activation in healthy adults.
Materials and methods: We measured BAT, subcutaneous adipose tissue (WAT) and skeletal muscle perfusion and GU using ^{15}O-H_2O- and ^{18}F-FDG-PET/CT in 26 healthy normal-weighted subjects during cold exposure and either with or without insulin stimulation using euglycemic clamp in normal environment. Energy expenditure with indirect calorimetry was assessed during PET/CT studies.
Results: During cold exposure, 70% of the subjects showed BAT activation. In those cases, GU in BAT was 10-fold increased (9.1±5.1 vs. 0.9±0.4 umol/100g/min, P=0.002). Perfusion in BAT correlated with GU rates in cold (r=0.82, P<0.001) but was only doubled (from 7.5±3.7 to 15.9±4.9 vs. ml/100g/min, P<0.001) and did not explain the activation. Insulin-stimulated GU in BAT was 5-fold higher than when measured at fast in normal room temperature (4.7±2.4 umol/100g/min, P<0.001). No association was found between insulin-stimulated BAT GU and perfusion. The effect of insulin on BAT metabolic rate was close to that in skeletal muscle (6.0±2.5 umol/100g/min) while GU in WAT was increased only by 50% by insulin. Plasma norepinephrine concentrations were increased substantially during cold exposure and energy expenditure tended to be higher among the subjects with active BAT.
Conclusions: Glucose uptake in BAT is under hormonal control and can be activated by insulin close to the same extent as in skeletal muscle in healthy adult humans. This increment of metabolism is independent on perfusion. Whether dietary and hormonal activation is altered in obesity is under evaluation.
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Effects of AMPK activation on glucose and energy homeostasis in leptin deficient *ob/ob* mice

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Background and aim: AMPK is a heterotrimeric complex, composed of a catalytic subunit (α) and two regulatory subunits (β and γ), which acts as a metabolic sensor to regulate glucose and lipid metabolism. A point mutation in the γ3 subunit (R225Q) increases basal AMPK phosphorylation, while concomitantly reducing sensitivity to AMP. AMPKγ3^{R225Q} transgenic mice have increased glycogen content, enhanced mitochondrial biogenesis and are protected against dietary-induced triglyceride accumulation and insulin resistance in glycolytic skeletal muscle. We determined whether skeletal muscle-specific expression of the AMPKγ3^{R225Q} isoform prevents metabolic abnormalities in leptin-deficient *ob/ob* mice (*ob/ob*-γ3^{R225Q} mice).
Materials and methods: Glucose tolerance and tissue-specific in vivo glucose uptake were determined in lean-wild-type (WT), lean-γ3^{R225Q}, *ob/ob*-WT and *ob/ob*-γ3^{R225Q} mice. Skeletal muscle glycogen and triglyceride content, as well as lipid oxidation was biochemically assessed.
Results: Glycogen content was increased in gastrocnemius muscle from lean-γ3^{R225Q} mice (lean-γ3^{R225Q} 5.3±0.4 mg/g vs. lean-WT 3.9±0.2 mg/g; p<0.05), as well as *ob/ob*-γ3^{R225Q} mice (*ob/ob*-γ3^{R225Q} 6.2±0.6 mg/g vs. *ob/ob*-WT 3.8±0.2 mg/g; p<0.05). Glucose tolerance was unaltered in either lean-γ3^{R225Q} or *ob/ob*-γ3^{R225Q} mice versus respective WT controls. Insulin-stimulated glucose uptake in EDL muscle during the euglycemic-hyperinsulinemic clamp was increased in lean-γ3^{R225Q} mice (p<0.05), but not in *ob/ob*-γ3^{R225Q} mice. In contrast, insulin-stimulated glucose uptake in soleus, brown adipose tissue (BAT) and heart was unaltered in lean γ3^{R225Q} and decreased (p<0,05) in *ob/ob*-γ3^{R225Q} mice. Basal lipid oxidation was increased in EDL muscle from lean-γ3^{R225Q} and *ob/ob*-γ3^{R225Q} mice (p<0.05). Pharmacological AMPK activation increased lipid oxidation in EDL, but not soleus muscle from lean-γ3^{R225Q} and *ob/ob*-γ3^{R225Q} mice. Elevations in gastrocnemius muscle triglyceride content were slightly prevented in *ob/ob*-γ3^{R225Q} mice. Whole body respiratory exchange ratio was unaltered between γ3^{R225Q} mutant and WT mice.
Conclusion: The AMPKγ3^{R225Q} mutation increases skeletal muscle glycogen content and enhances basal lipid oxidation in lean and *ob/ob* mice. However these improvements are insufficient to ameliorate insulin resistance and obesity in leptin deficient *ob/ob* mice. Thus, central defects due to leptin deficiency override any positive benefit conferred by peripheral overexpression of the AMPKγ3^{R225Q} mutation to improve glucose and energy homeostasis.

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Pancreatic roles for GLUT2 in human neonates evaluated by GLUT2 mutants

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Background and aims: GLUT2 is a facilitative sugar transporter; its low affinity and high capacity allow large fluxes of sugar in the liver, intestine, kidney and brain. GLUT2 is also a glucose receptor, detecting extracellular sugar and transducing a signal independent of glucose metabolism. The implication of GLUT2 in insulin secretion by beta pancreatic cells is well characterized in rodents but remains to be clarified in human. Mutations in human GLUT2 give a Fanconi-Bickel syndrome (FBS), patients suffer from glycogenosis and glucose homeostasis disorders but not from overt diabetes. A study showed that after oral glucose, young FBS patients displayed hyperglycemia and relative hypoinsulinemia, improving with age. Accordingly, GLUT2 expression in pancreatic beta cells, controversial in adults, was reported in human neonates. Neonates suffering from Congenital Hyperinsulinism syndrome (CHI) showed sever hypoglycemia due to high insulin secretion. Mutations in the pancreatic ATP-sensitive potassium channel are responsible for most characterized CHI, however 50% of cases remain unexplained and mutation in others genes are explored. Our hypothesis is that constitutive activation of GLUT2 functions could be consistent with CHI syndrome. The aim of this study was thus to evaluate the role of GLUT2 in human neonate pancreatic function investigating hGLUT2 mutants and their impact on insulin secretion.
Materials and methods: We generated by side-directed mutagenesis a panel of 6 hGLUT2 mutants. 3 single point homozygous mutations identified in FBS patients were generated to test their biological activity, they are suspected to abolish sugar transport. 3 other mutations were generated as possibly activating GLUT2 mutations (E,H,D). GLUT2 kinetic parameters were calculated by measuring the uptake of radio-labeled 2-deoxy-D-glucose in Xenopus oocytes. Insulin secretion was assayed using the insulin-secreting cell line Min6.
Results: As expected, the 3 FBS-associated mutations abolished GLUT2 transport function. Conversely, E and H mutations induced an increase in kinetic parameters of GLUT2 (lower Km, higher Vmax respectively) and D mutation decreased Vmax. Expression of these 3 mutants in Min6 cells stimulated insulin secretion at glucose concentrations inefficient in cells transfected with wild-type hGLUT2. For E mutant, insulin secretion occurred even in absence of glucose. This disqualified increased sugar transport as the single signaling event, and suggested that activation of receptor function of hGLUT2 may be in part responsible for the increased insulin secretion. Finally, we found in a pancreas sample of a CHI neonate that hGLUT2 was present in insulin positive cells, likely to play a crucial role on neonate human pancreas function.
Conclusion: With this work, we involve human GLUT2 as a direct actor in insulin secretion process not only through its transporter- but also through its receptor-function in neonates. Since activating mutations of GLUT2 can increase insulin secretion even in absence of glucose, we propose GLUT2 as a candidate gene to be sequenced in CHI patients. Modulating the receptor function of GLUT2 without affecting its transporter function that provides vital sugar may be a strategy to improve or decrease insulin production in patients suffering from metabolic diseases.
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OP 30 Insulin action and glucose uptake in vitro

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The effect of Astaxanthin, a strong antioxidant, on reactive oxygen species or insulin signaling *in vitro*

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Background and aims: Astaxanthin, a strong antioxidant, has been reported that it has a various effects on glucose or lipid metabolism, although precise mechanism(s) of its effect on signaling pathway in the cell remains to be elucidated. Here we examined its effects on reactive oxygen species (ROS) or insulin action *in vitro.*
Materials and methods: We stimulated L6 muscle cell or 3T3-L1 adipocyte with 50 mU/ml glucose oxidase (GO) or 7.5 mM Menadione, strong ROS generators widely used in research, or 10 nM insulin respectively, along with or without 30 μM Astaxanthin. The effect of Astaxaxnthin on signaling pathway in distinct conditions was conducted by western blotting, kinase assay or RT-PCR analysis.
Results: Astaxanthin enhances insulin-induced Akt phosphorylation but does not change IRS-1 phosphorylation. Intriguingly, it enhances GO or Menadione induced Akt phosphorylation as well although both generators do not phosphorylate IRS-1. We next explored which factor(s) involves in regulating further Akt phosphorylation by Astaxanthin. We found that PP2A activity, a serine/threonine-protein phosphatase, which dephosphorylates Akt, is subtly decreased by Astaxanthin itself and slightly decreased by insulin. On the other hand, Astaxanthin significantly lowered PP2A activity decreased by insulin. We speculate that Astaxanthin regulates Akt phosphorylation induced by insulin via regulation of PP2A activity and this effect requires insulin signal input. Similarly, we also observed that GO or Menadione decreased PP2A activity, again which is lowered by Astaxanthin. Although it has been reported that PP2A activity is regulated through src by insulin, src inhibitor does not have effect on enhanced Akt phosphorylation by Astaxanthin. These results suggest Astaxanthin regulates Akt phosphorylation induced by insulin or ROS generators through the same mechanism involving PP2A activity. Next, we explored the effect of Astaxanthin on cell apoptosis in various conditions. We found that Astaxanthin strongly decreased induction of Didt3 mRNA level or JNK phosphorylation by GO or Menadione. Furthermore, Astaxanthin decreased insulin induced JNK phosphorylation. Of particular interests to other molecules involved by insulin, shc phosphorylation is decreased with Astaxanthin. Finally we examined the effect of Astaxanthin on mitochondrial function since mitochondria plays an important role for oxidative stress response in the cell. We observed that Astaxanthin induced HO -1, PGC-1α or PPARα mRNA level significantly respectively.
Conclusion: These results *in vitro* suggest Astaxanthin appears to play a role as `insulin enhancer` and prevent cell from apoptosis induced by ROS presumably by eliminating ROS with regulating of mitochondrial function. Regarding intriguing Astaxanthin`s impact on insulin signaling, it seems to promote only good effect of insulin since shc phosphorylation leading to cell proliferation, which could cause some diabetic complication, induced by insulin is rather decreased by Astaxanthin. Similar results of the effect of Astaxanthin on insulin or ROS generators signaling pathway could be due to the ROS generation by insulin as reported. Astaxanthin would be a very effective tool which improves not only abnormal glucose metabolism or cell apoptosis caused by oxidative stress but also insulin sensitivity *in vivo* as well.

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Endothelin-1 acutely affects glucose metabolism in human skeletal muscle

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Background and aims: Endothelin (ET)-1 is a vasoconstrictor and pro-inflammatory peptide that may interfere with glucose uptake. The objective of the study was to investigate if exogenous ET-1 affects basal forearm glucose uptake in patients with insulin resistance (IR) and in cultured human skeletal muscle cells.
Materials and methods: Nine male subjects (age 61±3) with IR (total body glucose uptake <5.5 mg/kg/min or HOMA index >3) participated in a protocol using saline infusion followed by ET-1 infusion (20 pmol/min) for 2 h into the brachial artery. Forearm blood flow was assessed with venous-occlusion plethysmography. Endothelium-dependent (EDV) and -independent vasodilatation (EIDV) were determined. Forearm glucose uptake (FGU) was calculated from the arterio-venous plasma glucose concentration difference and plasma flow. Molecular signaling and glucose metabolism were determined in cultured skeletal muscle cells by western blot in the absence and presence of ET-1. Localization of ET receptors was characterized in human skeletal muscle tissue and cultured cells.
Results: Thirty min saline infusion did not change FGU. Infusion of ET-1 decreased FGU by 39% ($P<0.05$) from 5.8±2.0 to 3.4±0.8 μmol/min x 1000ml after 2 hour infusion. ET-1 administration decreased basal forearm blood flow by 36% (28.3±2.5 at baseline vs. 18.0±3.2 ml/min x 1000ml at 2 h of ET-1 infusion; $P<0.05$) and impaired both EDV ($P<0.01$) and EIDV ($P<0.05$). Incubation of cultured human muscle with ET-1 for 1 h increased glucose uptake in cells from subjects with normal glucose tolerance (NGT), but impaired insulin-stimulated glucose uptake in cells from IR subjects. ET-1 decreased insulin-stimulated Akt phosphorylation by 73% in NGT cells. ET_A receptor expression was detected in Western blots of cell cultures and in regions corresponding to the skeletal muscle cell membrane of skeletal muscle biopsies.
Conclusion: The study demonstrates that ET-1, in addition to attenuating endothelium-dependent vasodilatation, acutely impairs forearm glucose uptake in subjects with IR, as well as in skeletal muscle cells from IR subjects via a mechanism that seems to involve reduced Akt phosphorylation. This finding suggests that ET-1 may contribute to the development of IR.
Supported by: HLF, NNF

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Enhanced lipid -but not carbohydrate- supported mitochondrial respiration in PGC-1α overexpressing mice

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Background and aims: A reduced functional capacity of skeletal muscle mitochondria has been associated with type 2 diabetes mellitus. Both reductions in mitochondrial density and lower intrinsic mitochondrial function - i.e. respiration per mitochondria - have been reported in type 2 diabetic patients. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α), a master regulator of mitochondrial metabolism, has also been shown to be reduced in type 2 diabetes and muscle-specific overexpression of PGC-1α results in pronounced increase in mitochondrial density. Whether PGC-1α overexpression also results in intrinsic mitochondrial adaptations is currently unknown and subject of the present study.
Material and methods: To this purpose, we isolated skeletal muscle mitochondria from 10 male, ±10 week-old muscle-specific PGC-1α overexpressing mice (PGC-1αTG) and 8 wild-type (WT) mice. Equal mitochondrial quantities were then analyzed for their oxidative capacity by high-resolution respirometry, fuelled by a carbohydrate (pyruvate) and lipid (palmitoyl-CoA plus carnitine) substrate. Additionally, we assessed mitochondrial ROS production in isolated mitochondria by electron spin resonance (ESR) spectroscopy and UCP3 protein levels by western blotting.
Results: As anticipated, the mitochondrial yield was ~4.6-fold higher in PGC-1αTG mice as compared to WT mice (40.8 ± 3.4 vs. 8.9 ± 1.6 mg/g muscle, respectively; $p<0.001$). ADP-stimulated respiration fuelled by pyruvate tended to be reduced in mitochondria from PGC-1αTG (229.8 ± 11.9 vs. 260.1 ± 9.9 nmol O2/min/mg mitochondrial protein in PGC-1αTG vs. WT, respectively; $p=0.08$). Maximal uncoupled respiration, reflecting the maximal capacity of the electron transport chain was similar in both genotypes (396.5 ± 18.7 vs. 382.4 ± 17.0 nmol O2/min/mg mitochondrial protein in PGC-1αTG vs. WT, respectively; $p=0.60$). PGC-1αTG mice displayed a pronounced augmentation of mitochondrial fat oxidative capacity, evidenced by an increased ADP-stimulated respiration (136.9 ± 4.3 vs. 77.4 ± 2.6 nmol O2/min/mg mitochondrial protein in PGC-1αTG vs. WT, respectively; $p<0.001$). Also maximal uncoupled respiration was significantly enhanced in PGC-1αTG upon palmitoyl-CoA plus carnitine (159.4 ± 6.2 vs. 103.8 ± 3.6 nmol O2/min/mg mitochondrial protein in PGC-1αTG vs. WT, respectively; $p<0.001$). Mitochondrial ROS production was similar in PGC1αTG and WT

mice, and averaged 6705 ± 441 vs. 5933 ± 393 AU in PGC1αTG and WT, respectively (p=0.33). Mitochondrial UCP3 protein levels were markedly reduced in PGC1αTG mice as compared to WT (85.9 ± 14.2 vs. 24.8 ± 6.1 AU, respectively; p<0.001).
Conclusion: Besides stimulating mitochondrial proliferation in skeletal muscle, overexpression of PGC-1α also leads to clear intrinsic mitochondrial adaptations, i.e. an enhanced capacity upon fatty acids as substrates and a decreased UCP3 content. The low levels of UCP3 are in line with previous observations in endurance-trained athletes, who are also characterized by a high fat oxidative capacity.
Supported by: Dutch Diabetes Research Foundation

OP 31 Prevention of type 2 diabetes mellitus

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Vitamin D is associated with progression from impaired glucose regulation to type 2 diabetes in a UK multiethnic population
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Background: Vitamin D is implicated in the aetiology of Type 2 Diabetes (T2D). Cross-sectional analyses have consistently associated plasma 25-hydroxyVitamin D concentration (VD) with measures of insulin resistance and beta-cell dysfunction but prospective data relating to progression in groups at risk of T2D are scarce. Mixed ethnicity populations with a significant south Asian representation are an excellent study group as their rates of VD deficiency and T2DM are particularly high. Our aim was to determine if Vitamin D independently predicts progression to T2D within a UK multiethnic population with Impaired Glucose Regulation (IGR).
Materials and methods: In a population based screening study (ADDITION-Leicester), people with IGR, defined as a composite of WHO categorised Impaired Fasting Glycaemia (IFG) and/or Impaired Glucose Tolerance (IGT) are offered an annual 75g-Oral Glucose Tolerance Test and cardiovascular risk assessment. Baseline and one year measurements include standard anthropometrics, fasting and 2-hour glucose estimates, VD (IDS 25(OH) D2/D3 enzyme immunoassay), together with self-reported ethnicity, physical activity (via IPAQ questionnaire) and medication use (including non-propriety preparations). Those taking vitamin D or calcium supplements were excluded from this analysis. Baseline VD was adjusted for age, sex, waist circumference, physical activity and ethnicity. Logistic regression adjusting for confounders was used to identify if VD was independently associated with progression to T2D.
Results: 1,080 people with IGR were diagnosed from a total screened population of 6,749 (16% prevalence). 624 randomly selected subjects (75.6% White European, 23.9% South Asian,) with IGR but not taking VD supplements had VD analysed at baseline, of which 583 (93.4%) attended for follow up (Median duration 425 days, inter quartile range: 393 - 462). There were no significant differences amongst attendees and non attendees in terms of age, body mass index, blood pressure or glycaemic markers. 39 (6.7%) progressed to T2D, 225 (38.6%) continued to have IGR and 319 (54.7%) reverted to normal glycaemic status. Mean unadjusted baseline VD concentration for South Asians was 21.29±12.29 ng/l vs White Europeans 58±23.88 ng/l (p<0.001). Waist circumference (P<0.05), sex (p<0.05), physical activity (p<0.05) and ethnicity (P<0.001) influenced baseline VD concentration. Subjects progressing to T2D had a significantly lower adjusted baseline VD compared to those who continued to have IGR and who reverted to normal (T2D: 50.8±20.7 vs. IGR: 60.5±19.6 vs. Normal: 62.8±18.9 P=0.001). Lower adjusted VD significantly predicted progression to T2D at 12 months (Odds Ratios: 0.98, 0.96- 0.99, P=0.001). Significantly higher progression rates were seen in the lowest tertile of Vitamin D compared to higher levels (11.1% vs. 5.1% vs. 4.1% respectively, P=0.013), this difference remained statistically significant after adjustment for confounders.
Conclusion: Vitamin D may play an influential role in the progression of metabolic disease. Our preliminary data would support the need for a randomised intervention trial exploring the glucose lowering potential of VD replacement in multiethnic Northern latitude populations at risk of T2D.
Supported by: NovoNordisk Research Foundation

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Long-term outcomes from the PREPARE (Pre-diabetes Risk Education and Physical Activity Recommendation and Encouragement) randomised controlled trial
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Background and aims: The PREPARE programme, a theory-driven group-based structured education programme aimed at promoting increased am-

bulatory activity through pedometer use, has been shown to be effective at promoting physical activity and improving glucose regulation in people with impaired glucose regulation (IGT) at 12-months. The present study aims to assess whether the improvements in glucose regulation were sustained at 24 months.

Materials and methods: Overweight or obese individuals with IGT were recruited from population-based diabetes screening programmes, October 2006 to April 2007, Leicester, UK. Participants were randomized to receive either an advice leaflet, n = 34; a standard 3-hour group-based structured education programme aimed at promoting physical activity (E group), n = 31; or a 3-hour structured education programme that included enhanced self-regulation through personalized steps/day goals and pedometer use (EP group), n = 33. Both intervention conditions also received brief counselling at 3 and 6 months. The original study was designed to detect a 1 mmol/l difference in 2-h glucose; follow-up was conducted at 3, 6 and 12 months. As part of a local diabetes screening programme, participants were offered an additional appointment to assess their glycaemic status at 24 months. Outcomes included fasting and 2-hour glucose, progression to type 2 diabetes (WHO criteria) and body weight; all clinical measurements and blood assays were conducted blind to allocation using standardized criteria. Differences in change from baseline were analyzed using ANCOVA modelling.

Results: 24-month follow-up data was available for 74 (76%) participants; female = 38%, South Asian ethnicity = 20%. There was no difference in demographic or biochemical measures at baseline between those who did and did not attend. Those in the EP group maintained a significant reduction in 2-h glucose (baseline = 8.7 ± 2.3 mmol/l, follow-up = 7.3 ± 2.2 mmol/l; change compared to the control group adjusted for baseline value = -1.5 mmol/l; 95% CI = -2.8 to -0.3; p = 0.01). There was a trend towards a significant reduction in fasting glucose in the EP group (baseline value = 5.6 ± 0.6 mmol/l, follow-up = 5.4 ± 0.7 mmol/l, change compared to the control group adjusted for baseline value = - 0.3 mmol/l; 95% CI = - 0.7 to 0.0; p = 0.07). There was no difference between the control and E group in glucose levels. There was no difference in body weight in either intervention group compared to the control group. The accumulative incidence of type 2 diabetes over 24 months, diagnosed at any follow-up time-point, were: control = 18%, E = 16%, EP = 6%.

Conclusion: This study suggests that a 3-hour structured education programme aimed at promoting increased physical activity through personalized pedometer use leads to sustained improvements in glucose regulation in overweight or obese individuals with IGT, despite no change in body weight. This finding highlights the importance of promoting physical activity for its own sake rather than the end-point of weight loss. As the PREPARE programme is suitable for implementation within primary care, these findings are likely to have important implications for usual health care practice.

Supported by: Diabetes UK

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An individual lifestyle intervention program is not more effective in changing diabetes risk and lifestyle behaviors than providing health brochures: the Hoorn Prevention Study

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Background and aims: Diabetes mellitus type 2 (T2DM) is associated with lifestyle dependent risk factors. In this study we examined the effects of a lifestyle intervention targeting the lifestyle behaviors physical activity, diet and smoking in adults at high risk of T2DM, compared to providing written information only.

Materials and methods: Adults (n= 622) with an increased risk of developing T2DM based on the ARIC risk score were randomly assigned to the intervention or control group. The intervention group received a lifestyle intervention consisting of a cognitive behavioral program provided by trained practice nurses. In a maximum of six individual counseling sessions, followed by 3-monthly booster sessions by phone, *motivational interviewing* and *problem solving treatment* were used. The program focused in particular on intrinsic motivation to change and on self-management of problems. Primary outcome measure was the T2DM risk score with age standardized at 60 years. Secondary outcome measurements were physical activity, dietary behavior and smoking behavior. Results of the baseline and 6 and 12 months follow-up measurements are reported.

Results: 536 of the 622 participants (86.2%) completed the 6 months follow-up measurements and 504 completed the 12 months follow-up (81.0%). The mean age at baseline was 43.5 years (SD 5.3) and 363 participants were female (58.4%). The mean baseline risk score of the total sample was 18.9% (SD 8.2) on the ARIC risk formula. Participants in the intervention group received 2.5 counseling sessions on average. Regression analysis based on the intention to treat principle showed no significant differences in outcomes between the intervention and the control group at both follow-up measurements, adjusted for baseline (see Table 1).

Conclusion: The lifestyle intervention was not more effective at 6 and 12 months than providing written information, in improving T2DM risk score or lifestyle behaviors in an at risk population.

Table 1. Mean baseline and follow-up values (SD) and group differences corrected for baseline (95% CI) of T2DM risk score and lifestyle behaviors

	Control group			Intervention group			Group differences	
	Baseline	Follow-up 1 (6 months)	Follow-up 2 (12 months)	Baseline	Follow-up 1 (6 months)	Follow-up 2 (12 months)	β of between group difference Follow-up 1	β of between group difference Follow-up 2
Risk score								
ARIC	18.8 (8.5)	18.0 (7.6)	17.8 (8.2)	19.0 (7.8)	18.8 (8.5)	18.5 (8.3)	0.4 (-0.3 – 1.0)	0.3 (-0.8 – 1.6)
Physical activity[1]								
light activities	31.86 (17.3)	33.37 (18.2)	31.12 (16.7)	33.9 (17.8)	32.6 (16.5)	33.3 (17.4)	-1.5 (-4.2 – 1.2)	1.0 (-1.7 – 3.7)
moderate activities	10.6 (12.5)	10.5 (12.7)	11.7 (13.5)	11.4 (12.4)	10.1 (11.4)	10.3 (10.7)	-1.0 (-2.7 – 0.6)	-1.4 (-3.0 – 0.2)
vigorous activities	0.9 (2.4)	1.0 (2.0)	1.0 (2.4)	1.0 (2.2)	0.8 (1.7)	1.0 (2.2)	-0.2 (-0.5 – 0.1)	0.0 (-0.4 – 0.4)
Dietary behaviors								
fruit intake[2]	1.1 (0.8)	1.3 (1.0)	1.2 (0.9)	1.1 (0.9)	1.1 (0.9)	1.1 (0.9)	-0.2 (-0.3 – 0.0)	-0.1 (-0.2 – 0.0)
vegetable intake[3]	150 (70.4)	168 (276.6)	171 (184.1)	149 (71.4)	206 (547.6)	180 (300.6)	57.5 (-13.5 – 128.4)	9.6 (-37 – 56.2)
Smoking behavior								
smokers n (%)	55 (17.9)	46 (14.9)	43 (14.0)	75 (23.9)	53 (16.9)	46 (14.6)	OR 0.8 (0.3 – 2.0)	OR 0.6 (0.3 – 1.3)

ARIC= Atherosclerosis Risk In Communities formula based on: ethnicity (black yes/no), parental history of diabetes, systolic blood pressure, waist circumference and height;
[1] Data are based on responses to the Short questionnaire to assess health enhancing physical activity (SQUASH). Values are MET-hours per week, representing the average amount of time engaged in specified physical activities multiplied by the metabolic equivalent value of each activity. Light activities are rated as 2.0 to < 4.0 METs, moderate activities are rated as ≥ 4.0 to <6.5 METs, vigorous activities are rated as ≥ 6.5 METs
[2] Pieces of fruit per day
[3] Intake of vegetables in grams per day

Supported by: the Netherlands Organization for Health Research and Development

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Effect of a diabetes prevention programme (PREDIAS) on metabolic risk factors and quality of life: results of a randomised controlled trial

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Background and aims: The objective of this randomized, prospective trial was to evaluate the efficacy of a group program (PREDIAS) aiming at lifestyle changes and weight reduction for diabetes prevention with regard to weight reduction, glycaemic parameters and quality of life.
Materials and methods: The PREDIAS program consisted of 12 lessons based on the Diabetes Prevention Program. All lessons were delivered in group sessions. Topics of PREDIAS were: assessment of own diabetes risk, motivation for weight loss, behavioural strategies for weight reduction, physical exercise and stress management. The control group (CG) received written information about diabetes prevention. Risk factors like weight, fasting glucose and lipids as well as quality of life were assessed at baseline and at a 12 month follow-up. Quality of life was measured by using the Health Survey (SF12) consisting of 12 items.
Results: A total of 182 participants were randomised (age 56.3 ±10.1 yrs; 43% female; education 13.2 ±4.1 years; BMI 31.5 ±5.3 kg/m^2; fasting glucose 105.7 ±12.8 mg/dl). At follow-up 17 participants (9.3%) were lost to follow-up. At 12 month follow-up members of PREDIAS lost significant more weight than control group members (-3.8 ±5.2 vs. -1.4 ±4.0 p=.002). Fasting glucose decreased in PREDIAS by -4.3 ±11.3 mg/dl whereas it increased by 1.8 ±13.1 mg/dl in the control group (p=.001). There was no significant effect of the prevention program with regard to lipids (Cholesterol -10.3 ±35.9 vs. -2.0 ±35.1 mg/dl p=.144; Triglycerides -35.6 ±137 vs. -2.5 ±100.3 mg/dl p=.087, HDL -1.3 ±6.9 vs. -2.2±9.4 mg/dl p.479) and blood pressure (systolic RR -4.6 ±19.1 vs. -1.0 ±16.7 mm Hg; diastolic RR -4.4 ±11.7 vs. 2.1 ±12.6 mm Hg). At baseline all study participants reported a similar quality of life score in the SF 12 than the general population (Mental Component Summary Score (MCS) 50.3 ±5.3 vs. 51.1 ±8.1 p=.13: Physical Component Summary Score (PCS) 48.2 ±6.6 vs. 47.9 ±9.7 p=.36). However at 12 month follow-up members of PREDIAS reported a significant higher MCS than the control group (51.5 ± 3.9 vs. 49.9 ±5.4 p=.04). There was no significant effect of the prevention programme with regard to the PCS (50.7 ±5.6 vs. 48.3 ±5.7 p=.19).
Conclusion: The PREDIAS prevention program was able to reduce weight and fasting glucose significantly in a 12 month follow-up. The lifestyle change with regard to weight reduction was not achieved at the expense of quality of life aspects. Although other metabolic risk factors were considerable improved, the difference between members of PREDIAS and the control group failed to reach significance. The observed improvements with regard to metabolic risk factors after 12 month are comparable to meta-analytic findings about the efficacy of diabetes prevention programs. Since PREDIAS is a group intervention it can be expected that PREDIAS has the potential to provide lifestyle interventions effectively in a less expensive way than many hitherto evaluated diabetes prevention programs, which were mainly conducted in individual settings.
Supported by: Roche Diagnostics

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Effect of pioglitazone on beta cell function and adipocyte insulin resistance in impaired glucose tolerance: results form ACTNOW

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Background and aims: Individuals with impaired glucose tolerance (IGT) are maximally insulin resistant and have lost 70-80% of their beta cell function. Thiazolidinediones have been shown to delay/prevent onset of diabetes. ACT NOW is a randomized double-blind, placebo-controlled study to examine whether pioglitazone (PIO) can prevent/delay development of type 2 diabetes mellitus (T2DM). The aim of this study was to examine whether pioglitazone prevents/delays onset of diabetes by improving beta cell function.
Materials and methods: 602 IGT subjects (FPG =105, 2-h PG [OGTT]=168 mg%;) were randomized to PIO (45 mg/day) or placebo (PLAC) and followed for 2.8 years; 427 subjects returned for final study visit. Indices of insulin secretion and insulin sensitivity were derived from the plasma glucose, insulin, and C peptide concentrations during the OGTT. The acute insulin response ($AIR_{0\text{-}10}$ min) and insulin sensitivity (S_I) also were measured with frequently sampled intravenous glucose tolerance test (FSIVGTT) in a subset. Adipocyte insulin resistance was calculated as the fasting plasma FFA x fasting plasma insulin.
Results: 50 PLAC-treated subjects developed diabetes versus 15 PIO-treated subjects (hazard ratio=0.30, 95% CI=0.11- 0.54, p<0.00001). Pioglitazone therapy significantly reduced fasting and 2-h plasma glucose. Pioglitazone improved Matsuda insulin sensitivity index (MI) (4.3 ± 0.3 to 7.7 ± 0.3, p<0.0005). The insulin secretion insulin resistance index $\Delta I_{0\text{-}120}/\Delta G_{0\text{-}120}$ x MI (3.4 ± 0.3 vs 5.4± 0.3, p<0.0005) improved in PIO treated subjects. In contrast no significant changes in Matsuda Index (4.3 ± 0.3 to 5.2 ± 0.3, p=ns) or $\Delta I_{0\text{-}120}/\Delta G_{0\text{-}120}$ x MI (3.8 ± 0.3 vs 4.2 ± 0.2, p=ns) were observed in subjects treated with PLAC. Subjects treated with PIO also had significantly greater insulin secretion/insulin resistance index from FSIVGTT (Si x AIR) than PLAC (1186 ± 113 vs 832 ± 57, p=0.005). Pioglitazone reduced basal adipocyte insulin resistance index (5.96 ± 0.4 to 3.49 ± 0.4 p < 0.005) while no change in was noted in IGT patients on PLAC (5.97 ± 0.4 to 5.57 ± 0.4, p =ns).
Conclusion: Pioglitazone (1) improved β-cell function, and insulin sensitivity in IGT subjects, and (2) improved adipocyte insulin resistance. The improvement in beta cell function and adipocyte insulin sensitivity could represent fundamental mechanisms by which pioglitazone reduces the conversion of IGT to T2DM.
Supported by: Takeda Pharmaceuticals

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Lifetime health economic benefits of type 2 diabetes prevention in high risk subjects in an Australian setting: an updated analysis based on the results of the Diabetes Prevention Program and Diabetes Prevention Program Outcomes Study

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Background and aims: Metformin and intensive lifestyle interventions (ILI) were shown to reduce incidence of type 2 diabetes (T2D) versus standard care in overweight or obese subjects with impaired glucose tolerance (IGT) in the Diabetes Prevention Program (DPP) trial and Diabetes Prevention Program Outcomes Study (DPPOS), a total follow-up of 10 years. Our aim was to project the lifetime clinical and health economic outcomes to be expected from T2D prevention in high-risk subjects managed with standard care, metformin or ILI, based on the latest published results from the DPP+DPPOS.
Materials and methods: A semi-Markov, 2nd-order Monte Carlo computer simulation model was developed to project the 10-year clinical and resource utilization results of the DPP+DPPOS to patient lifetimes. Four health states were modelled: normoglycaemia (NG); IGT; T2D and dead. Subjects started in IGT and progressed to T2D or NG, at rates dependent on the treatment received. State-specific mortality rates for NG, IGT or T2D were used. We incorporated direct medical costs (from official Australian published sources and the reimbursement perspective) and Australian health utility and probability data. For each treatment arm, we calculated years free of T2D, cumulative incidences of T2D, non-discounted life expectancies, quality-adjusted life years (QALY), total lifetime costs and incremental costs per QALY gained versus standard care. Costs and QALYs were discounted at 5% annually. Univariate and probabilistic sensitivity analyses were performed.
Results: For standard care, metformin or ILI, mean (standard deviation) number of years free of T2D were 9.47 (0.08), 11.98 (0.09), 15.17 (0.11) years respectively. Cumulative incidences of T2D were 89.7% (0.2), 83.7% (0.2) and 73.4% (0.3%) for standard care, metformin or ILI respectively. Mean life expectancies from baseline age of 50 years were 27.64 (0.14), 27.95 (0.12), 28.33 (0.11) years for standard care, metformin or ILI respectively. Delayed onset of T2D led to QALY-gained of 0.12 (0.04) and 0.38 (0.05) years for metformin or ILI versus standard care, respectively. Total lifetime cost increases of $1,116 (4,338) per patient were projected for metformin versus standard care, and cost savings of $282 (4,222) for ILI versus standard care. ILI was therefore dominant to standard care, with both improvements in clinical outcomes and overall cost savings. Incremental costs per QALY gained for metformin versus standard care were $8,757. Probability of acceptance at a typical Australian willingness to pay threshold of $50,000 were 85% and 100% for metformin or ILI respectively. Results were most sensitive to probabilities of developing T2D, relative risks of mortality in each health state, and costs of implementing the interventions.
Conclusion: Based in the latest published data from the DPP+DPPOS, substantial improvements in lifetime clinical outcomes can be expected in high risk subjects treated with metformin or ILI to delay or prevent the onset of T2D. Prevention of T2D in this group of subjects is good value for money, and may even lead to long term cost savings in an Australian setting.

OP 32 Hypertension and heart failure

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The effect of combining angiotensin receptor blocker and direct renin inhibitor on albuminuria in type 2 diabetic patients with nephropathy

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Background and aims: Angiotensin receptor blocker (ARB) and direct renin inhibitor (DRI) have been shown to reduce albuminuria and preserve renal function in patients with diabetic nephropathy. Both agents are thought to confer renal protection via blockade of renin-angiotensin-aldosterone system (RAS). Simultaneous blockade of RAS at different levels with ARB and DRI may have synergistic anti-albuminuric effect compared to monotherapy. The study aims to compare the effect of combining Losartan and Aliskiren with that of administering either drug alone on 24-hour urine albumin excretion (UAE) in patients with type 2 diabetes mellitus (DM) with nephropathy.
Materials and methods: Forty-eight patients (mean age 58±9.2 years, 16 females) were prospectively studied. After a 4-week washout period, 23 patients received Losartan 100 mg once daily and 25 received Aliskiren 150 mg for 8 weeks. Following this, all 48 patients received a combination of Aliskiren 150 mg and Losartan 100 mg for 8 weeks, followed by a double dose of both drugs for another 8 weeks. Blood pressure (BP), glycosylated hemoglobin (HbA1c) and UAE were monitored.
Results: Baseline characteristics (age, BMI, duration of DM, HbAlc, BP, creatinine clearance and UAE) were similar in both groups. There was a significant reduction in mean (95% CI) UAE after 8 weeks of monotherapy [17.0% (4.2% to 31.8%) p=0.002]. The reduction in UAE was significant in patients treated with Losartan [23.8% (2.4% to 45.6%) p=0.01] but failed to reach statistical significance for patients treated with Aliskiren [11% (-7% to 29.1%) p= 0.076]. Mean BP reduction was not significantly different between these 2 groups (p= 0.66). After 16 weeks of combination therapy, there was further 11.4% reduction in UAE [(-8% to 31%), p = 0.018] while mean BP and HbA1c were not statistically different at the beginning (week 8) and end (week 24) of the combination therapy.
Conclusion: The study showed superior effect of Losartan (100 mg) over Aliskiren (150 mg) in reducing albuminuria in patients with diabetic nephropathy. The combination of both drugs showed further benefit in albuminuria reduction independent of BP control.

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Glycaemic and blood pressure variability correlates with cardiovascular factors in type 2 diabetic patients

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Background and aims: The correlation between Glycemic and Blood Pressure variability (GV and BPV) and cardiovascular risk has not been fully addressed.
Materials and methods: Therefore, the relationships between GV and BPV on one side and intima-media thickness (IMT), left ventricular mass index (LVMI), flow-mediated dilation (FMD) on the other side were evaluated in 26 DM2 patients (age 59±10 years; diabetes duration 53±58 months; HbA_{1c} 6.7±1.3%) on diet and/or metformin, no hypotensive treatment or complications. All subjects underwent 24-h continuous glucose monitoring: GV was calculated as Mean Amplitude Glucose Excursion (MAGE), Coefficient of Variation (CV), CONGA-1 and 2. From 24-h BP monitoring, CV systolic and diastolic BP (CV SBP and DBP) and the delta between nocturnal and diurnal BP [>10%: Dippers (D); <10%: Non-Dippers (ND)] were also calculated.
Results: IMT and LVMI were significantly increased in ND vs. D (0.77 ± 0.08 vs. 0.68 ± 0.13; p= 0.04 and 62 ± 23 vs. 50 ± 19 p= 0.047). All patients displayed a negative correlation between LVMI and delta SBP (r=-0.48 p= 0.02); while a positive correlation was observed with CONGA 1 and 2 (r= 0.54 p=0.005 and r= 0.65 p=0.0005, respectively). A negative correlation was observed between IMT and delta SBP and DBP (r= -0.42 p= 0.036 and r= - 0.54 p= 0.005, respectively) while such correlation was absent with GV indexes. Finally a negative correlation was found between CONGA 1 and FMD (r= -0.42 p= 0.032).
Conclusion: Our data show that glucose excursions and BP variability significantly impact on endothelial function and cardiovascular damage in patients with short duration of disease and optimal metabolic control.

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Different impact of type 2 diabetes mellitus and essential hypertension on aortic, carotid and peripheral vascular stiffness

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Background and aims: Diabetes and hypertension both accelerate vascular aging. Arterial stiffness an emergent biomarkers of cardiovascular disease, increases with age and in the presence of main cardiovascular disease risk factors, such as hypertension, diabetes and lipid disorders. Pathologic stiffening of large arteries with advancing age and risk factor exposure predominantly involves the elastic aorta and carotid arteries. Aim of this study was to evaluate the impact of type 2 diabetes, hypertension, and their combination on aortic, carotid and peripheral arteries stiffening.
Materials and methods: A total of 114 subjects were enrolled: 18 normotensive subjects (NT), 37 hypertensive individuals (HT), 20 diabetic normotensive (DMNT), and 39 diabetic hypertensive (DMHT). Applanation tonometry was used to measure aortic (carotid to femoral) and peripheral (carotid to radial) pulse wave velocity (aPWV and pPWV, respectively). Common carotid intima-media thickness (IMT) and carotid diameter were obtained by B-mode ultrasound image sequences, using the real-time computerized contour-tracking system "Carotid Studio". Common carotid stiffness (CCS) was determined from stroke change in lumen area and local pulse pressure obtained by applanation tonometry.
Results: Hypertensive groups (HT and DMHT) have similar systolic and diastolic blood pressure values and lipid parameters; diabetic subjects (DMNT and DMHT) have similar HbA1c levels and lipid profile. Peripheral pulse wave velocity (pPWV) was superimposable in all groups. On the contrary, aPWV significantly increased from NT (7.2±1.0 m/s) to HT (8.1±1.4 m/s) and DMNT (8.2±0.8 m/s), reaching the highest values in the DMHT group (10.6±1.9 m/s; Kruskal-Wallis, p<0.001). Common carotid stiffness (CCS) behaved similarly (NT 6.0±0.7 m/s, DMNT 6.5±1.2 m/s, HT 6.6±1.2 m/s, DMHT 7.3±1.2 m/s; Kruskal-Wallis, p<0.01). The presence of hypertension carried a higher risk of having increased (above the median value) aPWV (OR: 6.9; 5-95% confidence interval: 1.9-24.8), common carotid stiffness (OR: 2.8; 5-95%CI: 1.-1-7.4), and carotid diameter (OR: 3.9; 5-95%: 1.4-10.6), regardless of age and diabetes, while the differences were not significant for pPWV and IMT. The presence of diabetes carried a higher risk of having increased (above the median) aPWV (OR: 9.6; 5-95%CI: 3.3-27.2), and IMT (OR:2.7; 5-95%CI: 1.1-6.6), , regardless of age and hypertension, while the differences were not significant for pPWV, carotid diameter and common carotid stiffness.
Conclusion: Both type 2 diabetes and hypertension are associated with increased aortic PWV, and their combination induces an even greater aortic stiffening. Hypertension is characterized by vascular stiffening at both the aortic and carotid level. In contrast, type 2 diabetes is associated only with increased aortic PWV. The two conditions also differ for carotid remodeling characteristics, since hypertension determines carotid dilation while type 2 diabetes is associated with carotid wall thickening.

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Risk factor control in patients with type 2 diabetes and coronary heart disease

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Background and aims: Patients with type 2 diabetes have a markedly increased risk of coronary heart disease (CHD), and the long-term prognosis after a CHD event is worse in diabetic than in non-diabetic patients. We assessed risk factor control in patients with type 2 diabetes and CHD from the Swedish National Diabetes Register (NDR).
Materials and methods: Cross-sectional study of patients with first incidence of CHD (myocardial infarction, unstable angina, percutaneous coronary intervention and/or coronary artery bypass graft) 1-2 years before examination, aged 18-79 years: 666 examined in 2002, 1414 examined in 2005, and 2557

examined in 2008. HbA1c analyses were quality assured nationwide by regular calibration. Patients with LDL-cholesterol (LDL-C) data available were around 70% of all due to missing data. Significance was analysed by GLM regression, adjusting for age and sex.
Results: As shown in Table 1, mean HbA1c, blood pressure (BP) and LDL-C decreased across the 6-year period from 2002 to 2008, while mean BMI was unchanged and as high as 30 kg/m^2. Achievement of the treatment targets HbA1c <7%, BP ≤130/80 mmHg and LDL-C <2.5% improved considerably across the 6-year period, and were 56%, 48% and 79% in 2008. Patients examined in 2008 within intervals of HbA1c <7%, 7.0-7.9% and ≥8% were 56%, 27% and 17%, and those within intervals of systolic BP (SBP) <130, 130-139 and ≥140 mmHg were 36%, 26% and 38%. Use of antihypertensive drugs, aspirin (ASA), and especially lipid-lowering drugs increased across the 6-year period, and were as high as 96%, 87% and 90% in 2008. A high prevalence of adverse lifestyle characteristics prevailed during the 6-year period, and in 2008 the frequency of obesity (BMI ≥30 kg/m^2) was 44%, while 45% performed physical activity <3 times/week, and 20% of patients with age <65 years were smokers.
Conclusion: Control of HbA1c, BP and LDL-C improved significantly across the 6-year period from 2002 to 2008. Although fewer patients available in 2002, significant improvement was also seen for BP and LDL-C from 2005 to 2008. In 2008, treatment targets were achieved by more than half of patients for HbA1c, and by 70% for LDL-C. Although barely half achieved BP ≤130/80 mmHg, only one-third had systolic BP ≥140 mmHg. However, a high prevalence of adverse lifestyle characteristics prevailed. Evidence-based therapy with professional lifestyle intervention seems necessary for further improvement in secondary prevention.

Table 1. Risk factors in patients with type 2 diabetes and CHD

	Examination year			P trend	P 2005
	2002	2005	2008	2002-08	vs. 2008
Numbers	666	1414	2557		
Men, %	70	68	72	<0.01	<0.01
Age (mean), years	68 (8)	67 (8)	68 (8)	n.s.	n.s.
Duration (mean), years	10 (7)	9 (8)	9 (7)	<0.01	n.s.
HbA1c (mean), %	7.4 (1.3)	7.1 (1.1)	7.1 (1.1)	<0.001	n.s.
HbA1c <7.0, %	41.9	54.4	55.8	<0.001	n.s.
HbA1c 7.0-7.9 / ≥8.0, %	33 / 25	27 / 18	27 / 17	<0.001	n.s.
BP (mean), mmHg	139 / 76	138 / 75	134 / 74	<0.001	<0.001
BP ≤130/80, %	33.3	39.6	48.1	<0.001	<0.001
SBP <130 / 130-139 / ≥140	24/18/58	29/22/49	36/26/38	<0.001	<0.001
LDL-C (mean), mmol/l	2.6 (0.9)	2.3 (0.8)	2.2 (0.8)	<0.001	n.s.
LDL-C <2.5, %	48.5	65.4	69.6	<0.001	<0.05
BMI (mean), kg/m^2	29.6 (4.8)	29.6 (4.8)	29.7 (4.6)	n.s.	n.s.
Obesity (BMI ≥30), %	41.1	42.0	43.6	n.s.	n.s.
Phys. activity <3 per w, %	-	53.5	44.5	-	<0.001
Smoker + age <65 yrs, %	16.7	18.9	20.2	n.s.	n.s.
Antihypertensive drugs, %	88.5	94.4	95.7	<0.001	n.s.
Lipid lowering drugs, %	73.9	85.9	90.2	<0.001	<0.001
ASA, %	82.7	89.3	87.0	<0.001	n.s.

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Type 2 diabetes significantly modulates the impact of low left ventricular ejection fraction on the risk of cardiovascular events
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Background and aims: The prevalence of coronary artery disease and of congestive heart failure is high in patients with diabetes. In the present study we aimed at prospectively investigating the impact of the left ventricular ejection fraction (LVEF) and of angiographically verified coronary artery disease (CAD) on the risk of cardiovascular events in patients with type 2 diabetes (T2DM) and in non-diabetic subjects.
Materials and methods: Cardiovascular events were recorded over 8 years in 629 consecutive patients undergoing coronary angiography for the evaluation of established or suspected stable CAD. At the baseline angiography, significant CAD was diagnosed in the presence of significant coronary stenoses with lumen narrowing ≥50%, and the baseline LVEF was determined invasively by ventriculography.
Results: The baseline prevalence of significant CAD was higher (68.6% vs. 55.5%; p = 0.006) in patients with T2DM (n = 137) than in non-diabetic subjects (n = 492); the baseline LVEF was similar in these two patient subgroups (65±15% vs. 67±15%; p = 0.253). Prospectively, significant CAD (HR = 2.07 [1.50-2.88]; p <0.001) and the LVEF (standardised HR = 0.79 [0.71-0.88]; p <0.001) after multivariable adjustment both proved significantly predictive of caridiovascular events in a mutually independent manner. The incidence of vascular events was significantly higher in patients with T2DM than in non-diabetic subjects (43.8% vs. 30.1%; p = 0.003). In analyses with respect to the diabetes status, the LVEF strongly and significantly predicted cardiovascular events in non-diabetic subjects (HR = 0.72 [0.62-0.82]; p <0.001) but not in patients with T2DM (1.00 [0.75-1.22]; p = 0.711). An interaction term LVEF*T2DM was significant (p = 0.047), indicating that the cardiovascular risk conferred by a low LVEF was significantly higher in non-diabetic subjects than in patients with T2DM. The presence of significant CAD proved significantly and independently predictive of vascular events both in non-diabetic subjects and in patients with T2DM (HRs 1.84 [1.26-2.67]; p = 0.001 and 2.45 [1.18-5.06]; p = 0.016, respectively).
Conclusion: From the results of this 8-year prospective cohort study we conclude that T2DM significantly modulates the cardiovascular risk conferred by a low left ventricular ejection fraction.

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Predictors of incident heart failure in community-dwelling older adults with diabetes mellitus
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Background and aims: Type-II diabetes mellitus (DM) is a major risk factor for heart failure (HF). However, little is known about the risk factors for HF in those with DM. We used public-use copies of the Cardiovascular Health Study (CHS) datasets, obtained from the United States National Institutes of Health, to examine the predictors of HF in older adults with DM.
Materials and methods: Of the 5795 CHS participants, ≥65 years, 5461were free of baseline HF. Of these, 862 had baseline DM (based on past history and baseline fasting blood glucose ≥126 mg/dL) and 963 had baseline coronary artery disease (CAD). Multivariable-adjusted Cox regression models were used to determine predictors of centrally-adjudicated incident HF among those with DM during over 12 years of median follow-up. Considering that DM is considered CAD-equivalent, we repeated our analysis in a cohort with baseline CAD.
Results: Participants with DM had a mean (±SD) age of 73 (±5) years, 50% were women, and 24% were African American. Those with CAD had a mean (±SD) age of 74 (±6) years, 45% were women, and 15% were African American. Incident HF occurred in 272 (32%) and 324 (34%) of participants with DM and CAD respectively. Significant predictors of incident HF among those with DM and in those with CAD are presented in Table.
Conclusion: Community-dwelling older adults with DM had similar incidence and risk factors for new-onset HF as those with baseline CAD. Management of modifiable risk factors such as smoking and systolic blood pressure may provide opportunities for reducing risk of incident HF among both high-risk populations.

Table. Predictors of incident heart failure in older adults with diabetes mellitus (DM) and without DM but with coronary artery disease (CAD)

Variable	With baseline DM		With baseline CAD	
	Adjusted HR (95% CI)	P value	Adjusted HR (95% CI)	P value
Age 75 year and older	1.53 (1.8-2.00)	0.001	1.77 (1.41-2.23)	<0.001
Female	0.89 (0.69-1.15)	0.383	0.71 (0.55-.91)	0.008
Current smoking	1.55 (1.02-2.36)	0.038	1.77 (1.24-2.51)	0.001
Systolic blood pressure, mm Hg	1.02 (1.02-1.03)	<0.001	1.01 (1.00-1.01)	0.005
Baseline coronary artery disease	2.20 (1.68-2.88)	<0.001	---	---
Baseline diabetes mellitus	---	---	1.71 (1.22-2.39)	0.002
Serum creatinine, mg/dL	1.25 (1.06-1.48)	<0.001	---	---
Serum uric acid, mg/dL	1.13 (1.04-1.23)	0.003	1.14 (1.06-1.23)	0.001
Left ventricular systolic dysfunction	2.13 (1.50-3.04)	<0.001	1.54 (1.17-2.02)	0.002

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OP 33 HbA_{1c} for diabetes mellitus diagnosis: need for reassessment?

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A comparison of performance from using two HbA_{1c} cut-points (a 'rule-in, rule-out' spectrum) and one HbA_{1c} cut-point to detect type 2 diabetes in a multi-ethnic cohort

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Background and aims: HbA1c≥ 6.5% has been recommended as a diagnostic tool to detect people with Type 2 Diabetes Mellitus (T2DM). However, using HbA1c≥ 6.5% leads to discordance in people detected with T2DM from using an oral glucose tolerance test (OGTT). Therefore, using two HbA1c cut-points has been suggested to reduce the number of false positives/negatives: the first to 'rule out' T2DM (HbA1c ≤5.5%) and the second to 'rule in' T2DM (HbA1c≥ 7.0%). Those with HbA1c 5.6-6.9% could have T2DM, especially if 6.5-6.9%, and may need a further glucose test for diagnosis. The aim of this study was to compare detection rates for T2DM using (a) HbA1c≥ 6.5% or (b) the 'rule-out, rule in spectrum' and to determine the optimal cut-points in our multi-ethnic cohort.
Materials and methods: Analysis of 8696 previously undiagnosed primary care adults aged 40-75 years from the LEADER cohort, a combination of two systematic screening programmes. Participants underwent an OGTT and had HbA1c measured from 2002-2008 in Leicestershire, UK. T2DM was diagnosed according to WHO 1999 criteria.
Results: Use of an OGTT detected 291 (3.3%) people with previously undiagnosed T2DM. Using HbA1c≥6.5% to detect T2DM produced a sensitivity/specificity/positive predictive value (PPV)/negative predictive value (NPV) of 62.1%/97.7%/44.8%/98.9% in white Europeans and 78.9%/92.8%/36.2%/98.8% in south Asians. Using ROC curve analysis, the single optimal HbA1c cut-point for detecting T2DM was ≥ 6.1%, (sensitivity/specificity: 83.0%/87.8%) in white Europeans and ≥ 6.3% (sensitivity/specificity: 87.9%/85.5%) in south Asians. 'Rule-out, rule-in' spectrum: HbA1c ≤5.5% produced a high sensitivity/negative predictive value (NPV) of 98.4%/99.9% in white Europeans and 98.9%/99.7 in south Asians. HbA1c≥ 7.0% produced high specificity of 99.6% and 98.8% in white Europeans and south Asians respectively, however produced lower PPV of 76.0% and 68.1% respectively. Furthermore, 5115 (58.8%) people in the total cohort had HbA1c 5.6-6.9%, and 4793 (55.1%) people with HbA1c 5.6-6.4%. Using an alternate higher 'rule-out' cut-point of HbA1c ≤5.8% maintains a high sensitivity/NPV of 91.8%/99.6% in white Europeans and 97.9%/99.8% in south Asians; a lower 'rule-in' cut-point of HbA1c≥ 6.8% has a specificity/PPV of 99.4%/69.8% and 97.2%/53.6% in white Europeans and south Asians. The remaining spectrum of HbA1c 5.9-6.7% detects fewer people, n=2505 (28.2%).
Conclusion: The optimal HbA1c cut-point to detect T2DM studied was lower than HbA1c of 6.5% in both ethnic groups. Using a two cut-point 'rule-in, rule out' spectrum to detect T2DM appears to have better performance than using HbA1c≥ 6.5% in isolation in our multi-ethnic cohort. However, as over 50% of this cohort had HbA1c values between 5.6-6.9%, many people may require a subsequent glucose test on a second visit, involving fasting, which could be impractical to implement. Within our cohort, a better two cut-point spectrum of HbA1c ≤5.8% and HbA1c≥ 6.8% maintains high sensitivity/specificity/NPV and a reasonable PPV. Furthermore, using these cut-points, approximately one-quarter of the cohort would require a subsequent glucose test, which is more feasible to implement in clinical practice.

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Moving to the new HbA_{1c} diagnostic criteria has a deep impact on prevalence of gluco-metabolic abnormalities among high-risk Spanish population

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Background and aims: Diabetes screening in risk individuals is usually based on the detection of hyperglycemia through an increase in fasting plasma glu-

cose (FPG) or 2-hour post-load glucose (2hPG) in the oral glucose tolerance test (OGTT). The American Diabetes Association (ADA) has recently authorized the use of A1C as a diagnostic criteria for diabetes and other glucose abnormalities. To investigate the concordance between conventional 2hPG and/or FPG diagnostic criteria and the proposed new A1C criteria for diabetes, results from an active public health program (DE-PLAN) in Catalonia (Spain) were used.

Materials and methods: Non-diabetic individuals aged 45-75 y. were evaluated by general practitioners in 18 primary health care centres. They have been first screened using the FINDRISC questionnaire. A 2-hour oral glucose tolerance test plus A1C test (NGSP/DCCT rules) were simultaneously performed yearly.

Results: By January 2010 a total of 2287 blood test results have been recorded corresponding to 1144 subjects: 65% women; age=61.4 years; BMI=29.9 kg/m²; 68% with a FINDRISC score ≥12 points (moderate, high or very high risk). Diagnoses by 2hPG were: 1482 (64.8%) normal glucose tolerance (95% CI: 62.8-66.7), 609 (26.6%) prediabetes (20.7-25.6) and 196 (8.6%) diabetes (7.7-11.2). FPG-based findings were: 1572 (68.7%) normal FPG (66.8-70.6), 652 (28.5%) prediabetes (26.7-30.4) and 63 (2.8%) diabetes (2.2-3.5). Findings by A1C were: 1906 (83.4%) normal A1C *[<5.7%]* (81.7-84.8), 350 (15.3%) prediabetes *[5.7-6.4%]* (13.9-16.8) and 31 (1.3%) diabetes *[≥6.5%]* (0.9-1.9). Among the 201 diabetic diagnoses according to either the 2hPG or the A1C criteria, only 26 (12.9%) were classified as diabetic with both criteria (2hPG and A1C, *k* value=0.29). Likewise, only 19 out of 75 diabetic diagnoses according to either the FPG or the A1C criteria (25.3%) were classified as diabetic with both FPG and A1C (*k*=0.39). No differences in main phenotypes (age, sex, BMI, WC and risk score) were found between subjects who remained and did not remain diabetic by both criteria glucose and A1C. The overlap index between prediabetes diagnoses (2hPG / FPG vs. A1C) was 20.3%/27.2% (*k*=0.16/0.28, respectively).

Conclusion: To apply the new A1C diagnostic criteria evidenced a dramatic decrease in diabetes prevalence among this high-risk population by the FINDRISC. Overall, prediabetes and diabetes-specific concordances were very poor. A1C alone seems to be not advisable to screen for gluco-metabolic abnormalities among the high-risk Spanish population.

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Comparison of HbA_{1c} and OGTT in the diagnosis of diabetes in a high-risk population. The HUNT-DE-PLAN Study, Norway

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Background and aims: Due to more standardized methods of measuring HbA_{1c} and reports showing an association between diabetes complications and HbA_{1c} in persons without manifest diabetes, an HbA_{1c} of 6.5% has been introduced as a diagnostic criterion for diabetes. Those with HbA_{1c} 6.0-6.4% are recommended preventive measures. We wanted to compare the OGTT and HbA1c diagnostic criteria in persons defined at increased risk of diabetes.

Materials and methods: The third HUNT Survey was performed in 2006-2008, examining 50406 persons ≥20 years of age (54% of those invited). The population is almost exclusively Caucasian. All participants were asked to complete the FINDRISC questionnaire. In all 9.9% had a FINDRISC score of 15 or more corresponding to at least a 30% risk for diabetes in the next ten years. All defined in risk were invited to a follow-up study including an OGTT and HbA1c measurement. Glucose was measured in serum and HbA1c by a standard and continuously validated method, both at Levanger Hospital.

Results: In total 2645 persons participated in this follow-up study. The OGTT identified 254 (9.6%) with diabetes, 446 (16.9%) with impaired glucose tolerance (IGT) and 217 (8.2%) with impaired fasting glucose (IFG). Mean HbA_{1c} (SD) was 6.4 (0.7) for those with diabetes, 5.8 (0.5) for IGT and 5.8 (0.4) for IFG. The proposed new HbA1c diagnostic criterion defined 170 (6.5%) with diabetes and 17% (450) in the 6.0-6.4% zone. Of the 170 with HbA1c defined diabetes, 100 had OGTT defined diabetes and 167 of the 450 in the HbA1c risk zone had IGT/IFG.70 people had diabetes and 283 persons were at increased risk only by the HbA1c criterion, compared with to 154 with diabetes and 496 with IGT or IFG by the OGTT. Among those with diabetes according to WHO criteria 60.7% had an HbA1c below 6.5%.

Conclusion: The overlap between the 1999 WHO criteria and the proposed new HbA1c criterion for diabetes was poor in this geographically defined Caucasian population. The new criteria do not strictly define a risk zone, but the overlap between those with HbA1c 6.0-6.4% and those with IGT/IFG was also poor.

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Diagnosis of abnormal glucose levels in patients at high risk for the development of diabetes: A comparison of the oral glucose tolerance test and measurement of HbA_{1c} following the American Diabetes Association recommendations 2010

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Background and aims: American Diabetes Association have published in 2010 precise recommendations (ADA 2010) about (i) the population to be screened for dysglycemia, (ii) the diagnostic criteria for intermediate hyperglycemia (IH) and diabetes which include glucose values during oral glucose tolerance test (OGTT) and A1c and (iii) the patients to be considered for metformin treatment (those with both impaired fasting glucose and impaired glucose tolerance, or those with A1c ≥6%). The aim of the study was to evaluate diagnostic strategy with OGTT and/or A1C criteria.

Materials and methods: A total of 1157 patients (962 women; body mass index 37.0±7.2 kg/m²; 41.2±13 years old) fulfilling the ADA 2010 criteria to be screened and who had not been diagnosed for diabetes previously underwent an oral glucose tolerance test (OGTT) and measurement of A1c. They were assessed for diabetes risk score (Findrisc and DESIR score) and UKPDS coronary risk score.

Results: Based on OGTT and A1c respectively, 76 and 113 patients had diabetes; 307 and 299 patients had IH; and 130 and 255 patients would have been eligible for treatment with metformin. The sensitivity/specificity of A1c ≥6.5% for the diagnosis of diabetes according to OGTT were 45.9/92.0%. In patients with A1c <6.5%, the sensitivity/specificity of A1c 5.7-6.4% for the diagnosis of IH were 59.9/56.2%. Diabetes risk scores and UKPDS risk score were the highest in the 130 patients with both an abnormal OGTT and an A1c ≥5.7%.

Conclusion: OGTT and A1c are both considered as relevant diagnostic criteria for dysglycemia as they correlate with retinopathy and the risk for developing diabetes. We show in a population who should be screened that choosing the A1c strategy rather than the OGTT strategy leads to diagnose more diabetes and to treat more patients with metformin, although the consistency of both diagnostic criteria is low (for example, 1/3 of the patients with A1c ≥6.5% have a normal OGTT). The patients who have the highest *a priori* risk of diabetes and cardiovascular disease are those with an abnormal OGTT associated with an A1c≥5.7%.

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Hemoglobin A_{1c} in a population with pre-diabetes, diagnosed and previously undiagnosed diabetes

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Background and aims: Diabetes is a serious public health problem with epidemic characteristics. Diabetes diagnosis is based on the values of fasting plasma glucose and on the Oral Glucose Tolerance Test (OGTT). Recently, determination of A1c has been proposed for the diagnosis of "pre-diabetes" and diabetes. To analyze the reliability of screening for "pre diabetes" and undiagnosed type 2 diabetes using A1c values. To obtain information regarding A1c distribution in a representative population sample including subjects without diabetes, with diagnosed and previously undiagnosed diabetes, and with "pre diabetes".

Materials and methods: A randomized study was performed to determine the prevalence of diabetes in Portugal (PREVADIAB) covering 5,167 people randomly selected across the country, according to the population distribution. Diabetes prevalence was 11.7% between 20 and 79 years (previously diagnosed and undiagnosed). A1c was determined in all subjects. A1c data

were analyzed according to the recommendations of the "International Expert Committee Report on the Role of the A1c Assay in the Diagnosis of Diabetes" ADA/EASD/IDF (diabetes >= 6.5% and "pre-diabetes" > 5.7% and <6.4%).
Results: Excluding people who had a previous diagnosis of diabetes, 96.8% (CI 95%: 96.5% to 97.5%) of the population had A1c <6.5%. When the remaining 3.2% with A1c >= 6.5 were tested with an OGTT, 65% had undiagnosed diabetes, 29% had "Pre-Diabetes" and 6% had no diabetes criteria. 30,0% of previously undiagnosed people had A1c levels <6.5% (CI 95%: 23.6% to 36.4%). Thus, this method would not have allowed the diagnosis of 30,0% of cases of diabetes. Looking at metabolic control in people with previously diagnosed diabetes, we found that 34% had A1c values under 6.5%, 69.7% had values <7% and 15.4% had values > 8%. Analyzing A1c values in people with Impaired Fasting Glucose (IFG), 29.9% had values >= 6.5% and 98% values <7%, while in people with Impaired Glucose Tolerance (IGT) 29.7% had values >= 6.5% and 99.2% <7%. According to the criteria proposed by the ADA/EASD/IDF and analyzing the total population of PREVADIAB we verified that in the group of people with A1c values, <5.7%, 2.9% of people had diabetes. With A1c between 5.7% and 6.4%, 13.9% of people had diabetes and A1c values >= 6.4%, 86.5% of people had diabetes. "Pre-diabetes" was present in the three levels, mainly in the group with A1c values between 5.7% and 6.4% (30.0% of the group).
Conclusions: Using A1c > 6.4% as a means of diagnosis, a large number of people without previous diagnosis of diabetes (30,0%) would not be diagnosed. In the population with A1c values between 5.7% and 6.4% we found that 13.9% had diabetes, 30.0% had "pre-diabetes" and 56.1% had normal glycoregulation. Thus we should be cautious using A1c for diagnosis. In our opinion, the use of fasting glucose and OGTT remains pertinent.

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Discordance between fasting glucose-based and hemoglobin A_{1c}-based diagnosis of diabetes mellitus in Koreans

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Background and aims: Recently, the International Expert Committee recommended that hemoglobin A1c (HbA1c) ≥ 6.5% to be included as a diagnostic criterion for diabetes mellitus. However, the degree of diagnostic agreement with the fasting glucose-based criteria may be different across ethnic groups and populations. The aim of this study was to examine the difference between using fasting plasma glucose (FPG) and HbA1c criteria in screening for diabetes in Korean asymptomatic health check-up recipients.
Materials and methods: We retrospectively analyzed clinical and laboratory data of 37,754 Korean adults (age 20-89 years, 41% women) which were recorded during regular health check-ups. After excluding subjects with previously diagnosed diabetes mellitus (n = 1,812) and significant anemia or hemoglobinopathies (n = 318), 35,624 subjects (21,201 men and 14,423 women) were categorized by FPG (126 mg/dL) and HbA1c (6.5%) cutoff values.
Results: Among the 35,624 subjects without known diabetes, 1143 (3.2%) patients were newly diagnosed as having diabetes using FPG criteria and 1019 (2.9%) patients by HbA1c criteria (Table 1). Thus, using HbA1c alone could detect slightly less (-9%) diabetic patients compared with using FPG alone. By combining both FPG and HbA1c criteria, 1493 (4.2%) patients previously unknown to have diabetes could be newly diagnosed. Therefore, about 30% more diabetic patients could be detected by including new HbA1c criteria in addition to FPG criteria. Among the 1493 newly diagnosed diabetic subjects, 473 (31.6%) met FPG criteria only (DM-FPG group), 350 (23.5%) met HbA1c criteria only (DM-A1c group), and 668 (44.9%) were diagnosed as diabetes by both criteria. When we compared the DM-FPG and DM-A1c group, DM-A1c group were significantly older (55.1 ± 9.4 vs. 53.4 ± 9.2 years, $P = 0.032$), and had lower blood hemoglobin concentration (14.6 ± 1.5 vs. 15.2 ± 1.3 mg/dL, $P < 0.001$). In contrast, DM-IFG group had higher systolic (123 ± 15 vs. 119 ± 13 mmHg, $P = 0.006$) and diastolic (78 ± 9 vs. 74 ± 9 mmHg, $P < 0.001$) blood pressure, fasting serum insulin level (11.5 ± 8.7 vs. 8.8 ± 5.5 mIU/L, $P < 0.001$) and HOMA-IR (3.81 ± 2.95 vs. 2.47 ± 1.57, $P < 0.001$).
Conclusion: There was significant discordance in diagnosing diabetes mellitus between the FPG- and HbA1c-based criteria. Our results support the notion that fasting glucose criteria may be better in detecting patients with hepatic insulin resistance, while HbA1c criteria may be more useful in patients with predominant impairment of postprandial glucose metabolism. Further studies are needed to know which criterion is better in predicting the risk of long-term vascular complications of diabetes.

Table 1. Prevalences (%) of newly diagnosed diabetes mellitus in health check-up recipients according to age groups by fasting plasma glucose (FPG) and HbA1c criteria

	20-29	30-39	40-49	50-59	60-69	70-89	Total
FPG	0.4	1.5	2.8	3.9	4.7	7.7	3.2
HbA1c	0.3	1.4	2.3	3.5	4.8	6.4	2.9
Combined	0.5	1.8	3.4	5.1	6.9	10.7	4.2

OP 34 Inflammation in insulin resistance

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Effect of IL-1beta and TNFalpha inhibition on insulin secretion and metabolic control of type 2 diabetes patients with overweight or obesity

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Background and aims: To compare the effect of IL-1β and TNFα inhibition on insulin secretion and metabolic control of type 2 diabetes patients with overweight or obesity.
Materials and methods: A randomized, double-blind, placebo-controlled clinical trial was carried out in 40 type 2 diabetes patients, aged between 40 to 60 years, BMI between 25 to 34.9 Kg/m², glucose between 126 to 200 mg/dl, A1C >7%, without pharmacological treatment. At beginning and at end of the study, BMI, blood pressures, a metabolic profile (fasting glucose, A1C and lipids), IL-1β and TNFα concentrations were measured, as well as, insulin secretion assessment using the hyperglycemic - hyperinsulinemic clamp technique. The patients were randomly assigned to receive diacerein (50 mg twice/day), an inhibitor of IL-1β and TNFα, or placebo for a period of 60 days. Statistical analyses were calculated with Mann-Whitney U and Wilcoxon tests. The study protocol was reviewed and approved by the hospital-based Ethic Committee and written informed consent was obtained from all volunteers.
Results: In both groups decreased the BMI (30.9 ± 2.5 vs. 29.8 ± 2.5 kg/m², p = 0.002 and 30.6 ± 2.6 vs. 29.8 ± 2.8 kg/m², p = 0.001; respectively placebo and diacerein groups) in the same magnitude, p = 0.756. Diastolic blood pressure (77 ± 8 vs. 75 ± 6 mmHg, p = 0.040), fasting glucose (145 ± 28 vs. 124 ± 19 mg/dl, p = 0.001), A1C (8.3 ± 1.0 vs. 7.0 ± 0.8%, p <0.001), IL-1β (26.4 ± 6.6 vs. 17.9 ± 2.7 pg/ml, p=0.005), and TNFα concentrations (18.2 ± 3.9 vs. 13.8 ± 2.7 pg/ml, p=0.003) decreased significantly with diacerin administration, as well as increasing in first (17.0 ± 10.6 vs. 21.8 ± 12.7 μU/ml, p = 0.002), late (36.6 ± 18.6 vs. 46.9 ± 22.5 μU/ml, p = 0.002) and total (29.9 ± 15.4 vs. 36.1 ± 16.6, p = 0.002) phases of insulin secretion were observed.
Conclusion: Inhibition of IL-1β and TNFα by means of diacerein administration improved insulin secretion and the metabolic control of type 2 diabetes patients with overweight or obesity.

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CCR5 promotes adipose tissue inflammation and insulin resistance in obesity

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Background and aims: Adipose tissue macrophages (ATMs) play a critical role in obesity-induced inflammation and insulin resistance. Monocyte chemoattractant protein-1 (MCP-1) and its receptor CCR2 are important for ATM recruitment and the development of insulin resistance. However, deficiency of CCR2 or MCP-1 does not normalize obesity-induced ATM recruitment and insulin resistance. Therefore, other chemokine systems could also play a role in these processes. Recent reports have also shown that CCR5, a different CC chemokine receptor, and its ligands are upregulated in adipose tissue of human obesity. However, it is not known if CCR5 is involved in ATM recruitment and insulin resistance. Here, we investigated the role of CCR5 in obesity-induced adipose tissue inflammation and systemic insulin resistance by high fat (HF) feeding or leptin deficiency.
Materials and methods: We analyzed gene expression levels of several chemokines and their receptors in white adipose tissue (WAT) of genetically (ob/ob) and HF diet-induced obese (DIO) mice. To determine whether CCR5 is required for obesity-induced ATM recruitment and insulin resistance, we examined metabolic phenotype of CCR5-/- mice. In addition, we performed bone marrow transplantation (BMT) of CCR5-/- mice or wild type (WT) C57Bl/6J mice donor cells into irradiated WT recipient mice to generate myeloid cell specific chimeric mice.
Results: Expression of mRNA for CCR5 and its all ligands was markedly increased in WAT in both DIO mice (CCR5,11.1-; MIP-1α, 5.2-; MIP-1β, 5.2-; RANTES, 4.0-; MCP-2, 2.1-fold; all p<0.05 vs WT) and ob/ob mice (CCR5, 5.3-; MIP-1α,17.5-; MIP-1β, 19.9-; RANTES,4.9-; MCP-2,15.9-fold; all p<0.05 vs WT) at 15 weeks of age. Interestingly, upregulation of CCR5 and its ligands preceded ATM recruitment in DIO mice, and their expression levels were higher than MCP-1/CCR2 in ob/ob mice. CCR5-/- mice fed normal chow showed slightly better glucose tolerance. On a HF diet, CCR5-/- mice had decreased macrophage infiltration and crown-like structure formation in adipose tissue compared with WT mice at 20 weeks, even though weight and adipocyte size (191.5±9.3 vs 186.3±6.4 μm p=0.4) were similar. HF diet-induced insulin resistance and glucose intolerance were also significantly improved in CCR5-/- mice. These findings were associated with decreased inflammatory cytokine expression (TNFα and iNOS), reduction of endoplasmic reticulum stress evaluated by XBP-1 splicing, attenuation of MAPK (p38-MAPK, JNK) and NF-κB activation in adipose tissue, and improvement of hepatic steatosis. We next introduced CCR5 deficiency into ob/ob mice to generate double-knockout (DKO) mice. DKO mice were strikingly resistant to the development of both insulin resistance and fatty liver. DKO mice also had decreased ATM recruitment compared to ob/ob littermates. Importantly, mRNA expression for CCR5 and its ligands in adipose tissue was higher in stromal vascular fraction than adipocyte fraction from DIO mice at 15 weeks. Furthermore, BMT study revealed that chimeric mice lacking CCR5 in myeloid cells were protected from HF diet-induced hyperinsulinemia (CCR5-/-BMT 2.6±0.3 vs WT-BMT 5.1±1.1 ng/ml, p<0.05) and glucose intolerance.
Conclusion: Expression of CCR5 and its ligands is markedly increased in WAT of obese mouse models. Deficiency of CCR5 prevents insulin resistance induced by HF feeding or leptin deficiency. Therefore, CCR5 plays a crucial role in ATM recruitment and subsequent development of insulin resistance independently of MCP-1/CCR2.
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Heat shock protein 60 stimulates the secretion of pro-inflammatory adipokines from human adipocytes and induces insulin resistance in human skeletal muscle cells

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Background and aims: Adipose tissue is an endocrine active organ producing a variety of bioactive proteins. In the obese state expanded adipose tissue releases increased amounts of pro-inflammatory adipokines which mediate adipose tissue inflammation. It is well-known that obesity is a strong risk factor for the development of insulin resistance and a component of the metabolic syndrome which involves the crosstalk between adipose tissue and skeletal muscle. Adipokines released from enlarged fat cells are important mediators of this crosstalk by autocrine/paracrine and endocrine effects. The autologous stress protein heat shock protein 60 (Hsp60) is released from adipocytes and increased in serum of diabetic patients. This study is aimed to analyze the effects of Hsp60 on the release of adipokines from human adipocytes and to assess if Hsp60 affects insulin sensitivity of human skeletal muscle cells.
Materials and methods: Preadipocytes were isolated from subcutaneous adipose tissue of lean or overweight healthy women and differentiated *in vitro*. The release of pro-inflammatory adipokines after LPS and Hsp60 treatment was measured by a multiplex beads analyses. The specificity of Hsp60-binding to human adipocyte receptor structures was analyzed. *In vitro* differentiated skeletal muscle cells were incubated with Hsp60 concentrations ranging from 1 to 20 μg/ml. Insulin signaling and the induction of pro-inflammatory and stress pathways were analyzed by Western blotting.
Results: Unstimulated human preadipocytes and adipocytes secrete measurable amounts of TNFα, IL-6, IL-8, MCP-1 and RANTES. Hsp60 treatment leads to significantly increased secretion of TNFα (up to 168-fold), IL-8 (up to 7-fold) and RANTES (up to 9-fold) from preadipocytes as compared to untreated preadipocytes. As for mature adipocytes, Hsp60 stimulated the secretion of TNFα (up to 21-fold), IL-6 (up to 32-fold), IL-8 (up to 3-fold), MCP-1 (up to 6-fold) and RANTES (up to 8-fold) compared to unstimulated adipocytes. The specificity of Hsp60-Binding on human adipocytes could be demonstrated by binding assays. Binding of labeled Hsp60 could be inhibited by up to 69 % using unlabeled Hsp60, whereas ovalbumin was without effect. As human adipocytes express and release Hsp60, we tested the effect of this adipokine on skeletal muscle cells. Hsp60 activates pro-inflammatory and stress signaling in skeletal muscle cells in a dose-dependent way. Hsp60 significantly increases the phosphorylation of NF-κB, JNK and ERK1/2 up to 2-

to 3-fold over the control level. Hsp60 also induces insulin resistance on the level of insulin-stimulated Akt and GSK3 phosphorylation in a dose-dependent manner with significant inhibition being observed at concentrations from 5 to 20 μg/ml of Hsp60 which reflects pathophysiological concentrations.
Conclusion: We demonstrate for the first time that treatment of human preadipocytes and adipocytes with Hsp60 results in increased release of various pro-inflammatory adipokines in a dose- and differentiation-dependent way. In skeletal muscle cells Hsp60 activates pro-inflammatory signaling pathways and leads to impaired insulin signaling. Therefore, Hsp60 might be a factor contributing to adipose tissue inflammation and obesity-associated insulin resistance.
Supported by: DFG

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Communication between insulin-resistant skeletal muscle and beta cells
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Background and aims: Obesity and type 2 diabetes mellitus are characterized by an overlapping phenotype of insulin resistance with a relative deficiency in insulin secretion underlying hyperglycemia in type 2 diabetes. Systemic inflammation is another common feature, raising the hypothesis that elevated cytokine levels may contribute to peripheral insulin resistance and decreased beta cell functional mass. In healthy humans, TNF-α infusion induces skeletal muscle insulin resistance. We now explore the potential communication between insulin-resistant skeletal muscle and beta-cells.
Materials and methods: Human skeletal muscle cells were cultured for up to 24h with or without (control) 20 ng/ml TNF-α to induce insulin resistance. mRNA expression for cytokines was analysed after 8h by hybridising cDNA to low-density oligo-nucleotide arrays. Conditioned media (test: TNF-α-CM; control: C-CM) were collected and candidate cytokines were measured by antibody array. Human and rat primary beta-cells were used to explore the impact of exposure to conditioned media for 24h on apoptosis (TUNEL assay), proliferation (BrdU incorporation), short-term insulin secretion, and key signalling protein phosphorylation and expression. Data are mean+/-SE.
Results: Muscle cells (n=4) treated for 8h showed an increase in expression of 19 cytokine and chemokine genes (confirmed and quantified by RT-qPCR) with increased amounts of 13 cytokines after 24h in TNF-α-CM vs. C-CM. Both TNF-α-CM and TNF-α alone added to C-CM at the start of the beta-cell culture decreased glucose-stimulated insulin secretion to a similar extent. However, only TNF-α-CM also increased human and rat beta-cell apoptosis by 6.22+/- 2.1 and 6.14+/- 0.9 -fold respectively ($p< 0.05$) and decreased proliferation of rat beta-cells (1.08 +/- 0.15 vs. 8.2 +/- 1.7 % BrdU positive cells, TNF-α-CM vs. C-CM, $p<0.01$). In rat beta-cells, 1h of glucose stimulation (16.7 mM) induced phosphorylation of proteins from the insulin signalling pathway including Akt, AS160, other Akt substrates, and ERK, an effect prevented by prior culture for 24h with TNF-α-CM. Both TNF-α-CM and TNF-α added to C-CM decreased IRS-2 protein by approx. 60% but only TNF-α-CM also decreased IRS-2 mRNA by 42 +/- 5 % vs. C-CM ($p<0.05$). Interestingly, both CM and TNF-α-CM increased IRS-1 protein and mRNA expression. In order to identify which mechanism was involved in the specific effects of TNF-α-CM and potentially attributable to insulin-resistant muscle cytokines, rat beta-cells were pre-treated with GLP-1, IL1-Ra or IL6 blocker (AF-227-NA and Sant 7). Both GLP-1 and IL1-Ra partially protected against the TNF-α-CM evoked increased in apoptosis but not the decreased proliferation.
Conclusion: Taken together these results show that induction of insulin-resistance in human skeletal muscle by TNF-α leads to secretion of myokines which impact negatively on beta-cell proliferation and survival. The identification of these myokines and their molecular targets on beta-cells opens the possibility for new therapeutic strategies for preservation of functional beta-cell mass in type 2 diabetes.
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IL-6 stimulated TLR-4 gene expression via mTOR and STAT3 in human skeletal muscles myotube and human skeletal muscle of IGT subject
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Insulin resistance is associated with chronic inflammation, and many inflammatory cytokines and signaling pathways are involved. In this study we investigated the cytokines and mechanisms involved in the induction of insulin resistance in human skeletal muscle. We recruited 10 IGT subjects and 10 control subjects. Whole-body insulin-mediated glucose uptake was determined using a euglycemic hyperinsulinemic clamp test. Muscle biopsies were obtained from the vastus lateralis muscle. We determined levels of inflammatory cytokine, TLR gene expression, and insulin signaling using immunoblotting. We examined the mechanisms underlying TLR-4 gene expression using a human myotube culture system. Fasting blood glucose was significantly higher in IGT subjects than the controls. HbA1c showed a tendency to be higher in IGT subjects ($p=0.059$). Although there was no difference in HOMA beta cell function between the two groups, glucose utilization rates were significantly lower in the IGT group. Levels of IL-6, TNF-α, and TLR-4 was significantly increased in the IGT group, but TLR-2 was not. We studied which inflammatory cytokines induce TLR-4 gene expression using IL-6, TNF-α, free fatty acid, and high glucose. TLR-4 gene expression increased significantly in human skeletal muscle myoblasts treated with IL-6. To determine the main signaling pathway for IL-6-induced TLR-4 gene expression, we examined several signaling factors associated with IL-6 signaling pathways. We found that the active forms of signal transducer and activator of transcription3 (STAT3) was increased in the IGT group as compared with controls (mTOR: 183.22 ± 13.01 vs. 100 ± 12.63, $p < 0.05$; STAT3: 170.3949 ± 18.11 vs. 14.64, $p < 0.05$). Stattic (STAT3 inhibitor) markedly inhibited TLR-4 gene expression. We suggest IL-6 induction of TLR-4 gene expression via STAT3 is one of the main mechanisms underlying insulin resistance in human skeletal muscle.

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Toll-like receptor 2 knockout mice present iNOS-dependent insulin resistance
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Background and aims: There are evidences that the activation of JNK, IKK and iNOS pathways are associated with the reduction of the insulin sensitivity, but only recently it has been shown that those pathways can be integrated in the insulin resistance by membrane receptors, such as the Toll-like Receptors (TLRs). Studies in our laboratory show that mice with an inactivating mutation of TLR4 are protected from diet-induced obesity and activation of IKKβ and JNK. It is possible that other TLRs participate in this phenomenon. TLR2 is a good candidate, because it is activated by saturated fatty acids. However, no study has characterized the role of TLR2 in the insulin resistance of animal models. Therefore, the goal of the present study is to investigate the role of TLR2 on insulin resistance of mice.
Materials and methods: We investigated weight gain, insulin sensitivity and signaling in liver, muscle and white adipose tissue in TLR2 knockout (KO) mice and their controls, both fed with a standard chow. The glucose utilization was studied through euglicemic-hyperinsulinemic clamp, the protein signaling through Western Blotting, serum insulin, IL-6 and TNF-α through ELISA, oxygen consumption through an indirect open circuit calorimeter and glucose uptake by the soleus muscle was determined in vitro using 2-deoxy-D-[2,6-3H] glucose. In order to inhibit the expression of JNK and iNOS, we used SP600125 (30mg/kg body weight) and l-N6-(l-iminoethyl) lysine (l-NIL; 80 mg/kg body weight). All procedures were approved by the ethics committee at the Sytate University of Campinas.
Results: The animals were similar in concern to the weight gain, however TLR2 KO mice had a decreased oxygen consumption and a decreased UCP1 expression comparing with their controls. Moreover, TLR2 KO mice presented decreased glucose tolerance and decreased insulin sensitivity. The insulin signaling was also alterated in these animals, because the activation of the insulin receptor and of AKT was reduced. IKK activation was reduced in TLR2 KO mice, which was accompanied by the decreased serum concentra-

tion of IL-6 and TNF-α comparing with the controls, while the phosphorylation of JNK was increased in muscle and liver, suggesting that other proteins might be involved in the modulation of the insulin signaling, leading to an increased activation of JNK. In order to elucidate this question, we studied proteins associated with the endoplasmic reticulum (ER) stress, since they activate JNK, and observed an increased phosphorylation of PERK and an increased expression of IRE-1α, which are associated with the ER stress. However, when inhibiting the expression of JNK and iNOS, we observed that only the inhibition of iNOS was able to improve the insulin sensitivy, suggesting that the insulin resistance in these animals is iNOS-dependent.
Conclusion: Although we have found many activated mechanisms that have the potential of inducing insulin resistance in TLR2 KO mice, only one was capable of reversing this state - the iNOS pathway. Therefore, TLR2 KO mice present iNOS-dependent insulin resistance.
Supported by: FAPESP

OP 35 Novel aspects of beta cell function

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Fork-head box transcription factor, FoxO1 inhibits glucose-regulated insulin gene expression in pancreatic beta cells by direct binding to the promoter region
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Background and aims: Glucose and insulin stimulate preproinsulin (PPI) gene expression in pancreatic beta cells through mechanisms which are only partly defined. Pancreatic duodenum homeobox-1 (Pdx1) is a major transactivator of PPI whose action is antagonised by the fork-head box member protein, FoxO1. The effect of FoxO1 is thought to be largely due to inhibition of Pdx1 expression. However, we have also noted that a potential binding site also exists in the 5' flanking region of the rodent, and intron 2 of the human, PPI genes. Here we explore whether FoxO1 directly binds to this site to regulate insulin gene expression and examine the intracellular signalling pathways involved.
Materials and methods: Adenoviruses expressing wild type and a constitutively active (S256A) FoxO1-GFP and wild type Pdx1 were generated by standard techniques. Silencing of FoxO1 was achieved after 48 h of transfection of SiRNAs (10nM, Smart Pool) with Lipofectamine RNAiMAX in MIN6 beta cell lines. Chromatin immunoprecipitation was carried out after 24 h of CA-FoxO1 viral transduction, using a monoclonal anti-GFP antibody and CHIP grade protein G agarose. Real-time FoxO1 translocation was studied using a Nipkow spinning disc confocal microscope. The mRNA level was measured by qRT PCR using an ABI Fast Real-time PCR system normalised to endogenous cyclopholin. Insulin promoter-luciferase assays were carried out using a Stop and Glo, dual-luciferase kit. Statistical analyses of significance were done by Student's *t* test or ANOVA.
Results: Culturing of MIN6 beta cells at low (3mM versus 30mM) glucose led to a 2-3 fold decrease in PPI and Pdx1 mRNA levels. Constitutively active FoxO1-GFP over-expression led to a decrease in insulin and Pdx1 gene expression even at high glucose while silencing (~75%) of FoxO1 abolished the effects of low glucose. The effects of FoxO1 over-expression on PPI mRNA levels were still observed in the presence of over-expressed Pdx1 though both were present in the nucleus, and consistent with the direct action of FoxO1 on the insulin gene promoter. Chromatin immunoprecipitation using an anti-GFP antibody revealed direct binding of FoxO1 to a region located -768 to -141bp upstream of the transcriptional start site. FoxO1 over-expression inhibited the activity of an insulin promoter-reporter (luciferase) construct bearing this region and the latter inhibition was retained after co-expression of Pdx1, but lost in a truncated construct lacking the putative FoxO1 binding site. Confocal imaging revealed that wild type FoxO1-GFP translocated from the nucleus after 30-60 min of exposure to high glucose and this shift was blocked by a pharmacological inhibitor of phosphatidyl inositol 3' kinase (PI 3-kinase, LY294002) but not by inhibitors of glycogen synthase kinase 3 (GSK3 beta, SB216763 and SB415286).
Conclusion: We show here that FoxO1, which is regulated through a PI 3-kinase dependent signalling pathway, has a direct binding site on rodent insulin gene promoter. This newly identified mechanism may contribute to the regulation of endogenous Insulin and Pdx1 gene complimenting an effect on Pdx1 promoter described previously. Further dissection of this pathway may provide new therapeutic approaches to regulating the insulin gene in type 2 diabetes.
Supported by: Wellcome Trust, MRC, JDRF

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Carbohydrate-Responsive Element-Binding Protein (ChREBP) activity is regulated by Ca^{2+} ions in pancreatic beta cells via soluble resistant-related calcium binding protein (Sorcin)
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Background and aims: We have recently shown that ChREBP is an important mediator of pancreatic beta cell glucolipotoxicity. ChREBP activation by

high glucose increases the expression of the lipogenic genes liver-type pyruvate kinase (L-PK) and fatty acid synthase (FAS) and inhibits the expression of ARNT/HIF1-β . We have also shown previously that Ca^{2+} influx is necessary for ChREBP activation in these cells. However, the precise molecular mechanism(s) through which ChREBP activity may be regulated by Ca^{2+} is unknown.
Materials and methods: A yeast two-hybrid screen was performed using ChREBP as a bait and the Matchmaker Gal4 Two-Hybrid System 3 (Clontech) with an in-house MIN6 pancreatic β cell library. Mammalian cell transfection was carried out using Lipofectamine 2000, and cells were imaged using a Zeiss Axiovert 200M microscope fitted with a PlanApo x63 oil-immersion objective. Confocalilty was achieved using a Nipkow spinning disc under the control of Volocity™ 4.0 (Improvision) software. An EGFP-ChREBP chimaera was generated by in-frame fusion of the corresponding cDNAs. Sorcin-specific siRNAs were purchased from Dharmacon. Chromatin immunoprecipitation was carried out using an in-house rabbit polyclonal anti-ChREBP antibody.
Results: Sorcin, a penta EF hand Ca^{2+} binding protein, was identified as a ChREBP interacting partner by yeast two-hybrid analysis. We confirmed that sorcin and ChREBP interacted in INS-1(832/13) and MIN6 β cells by co-immunoprecipitation and that they also co-localised in the cytosol in a punctiform pattern in cells maintained in 3 mM glucose, as revealed by confocal microscopy. However, the extent of ChREBP and sorcin colocalisation was markedly reduced when cells were maintained at elevated (30 mM) glucose concentrations, where ChREBP staining became apparent in the nucleus. Moreover, ChREBP binding to the L-PK promoter, assessed by chromatin immunoprecipitation, was increased at low glucose concentrations following sorcin inactivation by RNA interference. As the apparent physical interaction of sorcin with ChREBP implied that ChREBP may be regulated by intracellular Ca^{2+}, we tested the ability of ChREBP to respond to elevated intracellular levels of these ions. Using live cell imaging of a GFP-tagged ChREBP construct, we found that ChREBP translocated into the nucleus within 5 - 7 min. of cell depolarization with 50 mM K^+ and activated Ca^{2+} influx. Finally, demonstrating the likely importance of sorcin in retaining ChREBP in the cytosol, sorcin silencing significantly inhibited insulin secretion from MIN6 β cells.
Conclusion: These results demonstrate that sorcin is a physiologically relevant molecular binding partner from ChREBP, and define a role for sorcin in β cell function and insulin secretion. We propose a model wherein, at low glucose concentrations, sorcin sequesters ChREBP in the cytosol. Elevated glucose concentrations, which trigger Ca^{2+} influx, lead to conformational changes in sorcin which release ChREBP and allow it to translocate into the nucleus to regulate the transcription of genes involved in lipid synthesis. The up-regulation of these target genes may then contribute to gluco-lipotoxicity, and hence diminished beta cell function and survival, in the context of type 2 diabetes.
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Beta cell specific c-Kit receptor over-expression improves beta cell growth and function
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Background and aims: One of the major defects in diabetes is the loss of insulin producing cells in the pancreas. The reasons for beta-cell loss are not well understood. We previously focused on determining factors responsible for the maintenance of beta-cell mass and function and have demonstrated that the c-Kit receptor and its ligand, stem cell factor (SCF), are important for both rodent and human pancreatic islets. These results show that c-Kit is not only a marker of beta-cell precursors but is also critical for beta-cell proliferation, maturation, function and survival *in vitro*. Study on the *c-Kit*$^{Wv/+}$ mice further showed that the mutant male mice displayed early onset of diabetes. However, a better understanding of the underlying mechanisms is necessary prior to the development of novel physiologically relevant cell-based approaches to treat and manage diabetes. The aim of the present study is to examine whether a beta-cell specific c-Kit overexpression would have physiological and functional implications in beta-cell development and function.
Materials and methods: A beta-cells specific over-expression c-Kit [RIP-c-Kit(h)] transgenic mouse model in *C57BL/6J* background was generated followed by the characterization of beta-cell proliferation and function.
Results: We found that the beta-cell specific c-Kit transgenic mice display relatively large body mass and normal fasting blood glucose level compared to the control littermates. However, a significant improved glucose tolerance in the beta-cell c-Kit transgenic mice at both 4 and 8 weeks of age was observed ($p<0.05$). Morphometric analysis revealed a significant increase in the islet number and size ($p<0.01$) at 4 weeks of age. Increase beta-cell mass in beta-cell c-Kit transgenic mice along with an increase in beta-cell proliferation, Pdx-1 and Nkx6.1 expression compared with controls ($p<0.05$).
Conclusion: Our results demonstrate that c-Kit receptor tyrosine kinase is involved in the regulation of glucose metabolism and contributes to the maintenance of beta-cell function.
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Mitochondria distinguish between fast and slow cytosolic Ca^{2+} oscillations in pancreatic beta cells
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Background and aims: The normal pulsatility of insulin secretion from pancreatic beta cells, critical for maintenance of glucose homeostasis, is lost in type 2 diabetic patients. Mitochondrial Ca^{2+} uptake has been suggested to be involved in the control of oxidative metabolism and ATP generation which, via the closure of ATP-sensitive K^+ channels, may underlie fluctuations in the electrical activity of the beta cell plasma membrane. Whether Ca^{2+} influx into mitochondria can occur rapidly enough to contribute to the polarisation of the plasma membrane during glucose-induced bursts of electrical activity, or to slower oscillations in plasma membrane potential (V_m), is unknown. Here, we have combined optical imaging of targeted probes and patch clamp electrophysiology to monitor cytoplasmic $[Ca^{2+}]$ ($[Ca^{2+}]_c$), intramitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_m$), and V_m simultaneously in single living beta cells. The relationship between these parameters was studied in response to glucose or other stimuli.
Materials and methods: Mouse beta cells were infected with an adenovirus encoding the ratiometric GFP-based mitochondrial Ca^{2+} sensor, pericam. V_m was manipulated using the perforated-patch patch-clamp technique. The dynamics of Ca^{2+} changes in the cytosol (Fura-Red) and mitochondria (pericam) were imaged simultaneously using appropriate excitation wavelengths (490 and 410 nm respectively) and emission filters.
Results: Glucose (17 vs 3 mM) induced slow (2-5 min period) oscillations in $[Ca^{2+}]_c$. The onset of each oscillation was tracked by changes in $[Ca^{2+}]_m$, with the changes delayed by ~10s. Each glucose-induced increase of $[Ca^{2+}]_c$ was preceded by depolarization of the plasma membrane. The imposition of depolarizations by extracellular K^+ was able to mimic the glucose-induced oscillations of Ca^{2+} in both compartments, indicating that mitochondrial Ca^{2+} uptake occurred as a result of the $[Ca^{2+}]_c$ increase. Likewise, acetylcholine-mediated Ca^{2+} mobilization from intracellular stores prompted increases in $[Ca^{2+}]_m$ and these were also delayed compared to the $[Ca^{2+}]_c$ increases. For a given $[Ca^{2+}]_c$ rise, peak $[Ca^{2+}]_m$ values were larger after cell membrane depolarization than after Ca^{2+} mobilization. To explore whether $[Ca^{2+}]_c$ changes elicited by rapid membrane depolarizations could be sensed by mitochondria we next imposed trains of action potentials, mimicking those provoked by glucose, by voltage clamping the cell membrane. Such trains (23 pulses to 0 mV in 6 s imposed in 15 s intervals) led to increases in $[Ca^{2+}]_c$, which were maximal after the first burst, and partially recovered between bursts. By contrast, $[Ca^{2+}]_m$ did not increase detectably until the third burst in this protocol, and did not increase at all when the interburst interval was increased to >30 s.
Conclusion: We show that Ca^{2+} accumulation by beta cell mitochondria is dependent upon the duration and frequency of $[Ca^{2+}]_c$ pulses. These results suggest that: (a) the filtering out by mitochondria of $[Ca^{2+}]_c$ oscillations with sub-threshold frequency may contribute to the steep dependency of insulin secretion upon glucose concentration; (b) mitochondrial Ca^{2+} uptake and release are not a prerequisite for plasma membrane bursting electrical activity; (c) deranged uptake of Ca^{2+} by mitochondria may contribute to defective insulin secretion in some forms of type 2 diabetes.
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Impaired pancreatic beta cell Ca^{2+} dynamics and function in premature ageing

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Background and aims: People over age 65 have a significantly increased risk for type 2 diabetes. In ageing humans, only those whose beta-cells fail to compensate for insulin resistance develop diabetes. However, the ageing of beta-cells is poorly understood. Here we, for the first time, demonstrates a clear coupling between ageing and impaired pancreatic beta-cell function and thereby glucose homeostasis in mice with premature ageing induced by accumulation of mitochondrial DNA (mtDNA) mutations.
Materials and methods: We used homozygous knock-in mice expressing a proofreading-deficient form of the catalytic subunit of mtDNA polymerase γαμμα. This mutant polymerase γαμμα induces error-prone mtDNA synthesis, which, in turn, leads to accumulation of somatic mtDNA mutations with increasing age and a reduced lifespan and premature onset of ageing-related phenotypes. We performed glucose and insulin tolerance tests in animals. Cytochrome c oxidase and succinate dehydrogenase activities were measured by enzyme histochemical double staining of cryostat sections from pancreas. In isolated islets, measurements of glucose-stimulated changes in insulin release, mitochondrial membrane potential and cytoplasmic free Ca2+ concentration ([Ca2+]i) were performed.
Results: Compared to wild-type littermate control mice, the mtDNA mutator mice develop impaired glucose tolerance ($p<0.05$) due to progressive mitochondrial respiratory chain dysfunction in pancreatic islets and subsequent deficiency in glucose-stimulated insulin secretion ($p<0.01$). Both δελτα rhodamine 123 fluorescence, a probe to measure mitochondrial membrane potential, and δελτα peak [Ca2+]i values were decreased in islets obtained from mtDNA mutator mice after stimulation by 11 mM glucose ($p<0.001$ and $p<0.01$). We further demonstrate that the progressive decline in pancreatic beta-cell function in mice with premature-ageing phenotypes is associated with a slower frequency ($p<0.05$) and decreased amplitude ($p<0.001$) of glucose-stimulated oscillations in [Ca2+]i. The latter findings are likely to be accounted for by down-regulated PLC/InsP3-mediated Ca2+ mobilization from intracellular stores and decreased beta-cell Ca2+ influx over the plasma membrane.
Conclusion: This study demonstrates that an age-related deterioration in [Ca2+]i signaling serves as a link between impaired beta-cell function and premature ageing in mtDNA mutator mice. It also suggests that enhancement in beta-cell Ca2+ signaling can be a potential target for the development of novel pharmacological regimens in the treatment of diabetes in the ageing population.
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Loss of anti-apoptotic Bcl-x_L or Bcl-2 enhances beta cell glucose signalling

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Background and aims: In many cell types, including pancreatic beta-cells, Bcl-2 and Bcl-x_L have been demonstrated to preserve mitochondrial integrity and suppress apoptosis under a variety of cellular stress conditions. Whether these important pro-survival proteins also regulate beta-cell physiology in vivo or in vitro under normal conditions is not known. In this study we used a combination of genetic and pharmacological loss-of function approaches to test the hypothesis that Bcl-2 and Bcl-x_L impact beta-cell energy metabolism, calcium homeostasis and insulin secretion.
Materials and methods: Beta-cell function and glucose homeostasis were studied in vivo and following conditional deletion of Bcl-x in adult beta-cells by tamoxifen-injection of Bcl-$x^{flox/flox}$;Pdx1-CreER (Bcl-x KO) and littermate Bcl-$x^{flox/flox}$ (Bcl-x WT) control mice. Glucose signalling was assessed in islets and dispersed beta-cells from Bcl-2 knockout, heterozygous and wild-type littermate mice. Small molecule antagonists were used to assess the impact of acute Bcl-2/Bcl-x_L inhibition on mitochondrial physiology, cellular ATP/ADP, calcium signalling and insulin secretion of beta-cells in vitro.
Results: Quantitative real-time PCR and western blot confirmed the near complete loss of Bcl-x_L expression and protein in islets from inducible Bcl-x KO mice. Loss of islet Bcl-x resulted in a moderate improvement of glucose tolerance in 10-12 week old mice within days of tamoxifen administration. The cellular ATP-to-ADP ratio of Bcl-x KO islet cells was markedly increased in the presence of both basal and stimulatory glucose. Moreover, cytosolic calcium responses were significantly enhanced in glucose-stimulated islets and dispersed beta-cells from both Bcl-x_L and Bcl-2 deficient animals relative to their respective controls. In accordance with these findings, acute treatment of normal mouse and human islet cells with Bcl-2/Bcl-x_L antagonists in the presence of 3 mM glucose enhanced basal glucose-dependent respiration and induced mitochondrial calcium fluctuations within minutes. This raised ATP/ADP and triggered K_{ATP} channel- and voltage-dependent calcium influx and insulin secretion. Sustained Bcl-2/Bcl-x_L inhibition resulted in beta-cell death but detailed time-course analyses demonstrated the induction of apoptosis to be temporally and causally disconnected from the observed physiological responses.
Conclusion: Our findings demonstrate that anti-apoptotic Bcl proteins exert a tonic suppression of beta-cell metabolic signalling and thus work at the interface of beta-cell survival and physiology. Further study of these survival-regulating proteins and the molecular mechanisms of their metabolic functions may help identify factors to preserve the functional beta-cell mass required to maintain glucose homeostasis.
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The endocannabinoid system links gut microbiota to adipogenesis

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Background and aims: Obesity is characterized by a massive expansion of the adipose tissue associated with a low-grade inflammation. Recently, we and others have proposed that gut microbiota would favour the occurrence of inflammation, insulin resistance and metabolic diseases associated with obesity. We have demonstrated a connection between the gut microbiota, fat mass, gut permeability and inflammation associated with higher plasma LPS levels (metabolic endotoxaemia). The endocannabinoid-system (eCB) plays a major role in the development of the inflammation and metabolic disorders associated with obesity via mechanisms not fully understood. Interestingly, LPS is known to stimulate eCB system tone. Therefore, we postulate that the higher plasma LPS levels and eCB system-tone found in obesity could act as key mechanisms leading to gut barrier disruption and altered adipogenesis.
Materials and methods: To determine the contribution of gut microbiota on the regulation of the intestinal and adipose tissue eCB-system tone (CB_1 mRNA, FAAH mRNA, AEA) in both physiological and obese conditions, we investigated selective (prebiotics, high-fat), drastic (antibiotics, germ-free mice) modulation of the gut microbiota and bacteria-host interaction (*Myd88*$^{-/-}$) models. To investigate the role of the eCB-system tone, we blocked the CB_1 receptor with a specific and selective antagonist (SR141716A) in obese *ob/ob* mice, or we mimicked the higher eCB-system tone observed during obesity by chronic (4-weeks) infusion of a CB receptor agonist (HU-210). We investigated *in-vivo* and *in-vitro* intestinal permeability, adipocyte differentiation (PPAR-γ, aP2, C/EBP-α) and lipogenesis (SREBP-1c, ACC, FAS) and the occurrence of inflammation (plasma LPS, cytokines) in the different models.
Results: Obese mice (genetic and nutritional models) are characterized by a higher intestinal and adipose tissue eCB system tone (higher AEA content and CB_1 mRNA expression, lower FAAH mRNA). We found that the gut microbiota directly controls the intestinal eCB system-tone in all five models of gut microbiota modulation. We found *in-vitro* and *in-vivo* that the activation of the intestinal eCB-system increases gut permeability (higher plasma LPS and plasma Dextran-FITC, alteration of tight junctions proteins ZO-1 and Occludin). We demonstrated that the blockade of the CB_1 receptor reduced plasma LPS levels by a mechanism linked to the improvement of these markers. At the adipose tissue level, we show both *in-vitro* and *ex-vivo* that both eCB system and gut microbiota regulates adipogenesis, by increasing markers of differentiation (PPAR-γ, aP2, C/EBP-α) and lipogenesis (SREBP-1c, ACC, FAS). In addition, we found that LPS acts as a key regulator on the endocannabinoid and PPARγ-driven adipogenesis.
Conclusion: First, we demonstrate that the peripheral (intestine and adipose tissue) eCB-system tone is under the control of the gut microbiota. Second, we demonstrate that eCB-system controls gut barrier function and therefore endotoxaemia. Third, we provide evidence that adipogenesis is under the control of the gut microbiota, through the modulation of the gut and adipose tissue endocannabinoid systems. These data indicate that gut microbiota determines adipose tissue physiology through LPS-eCB system regulatory loops and may play a critical role in the adipose tissue plasticity during obesity.
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Depot-specific induction of transdifferentiation, inflammation and apoptosis via cannabinoid type 1 receptor blockade

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Background and aims: The endocannabinoid system is a major component in the control of energy metabolism. CB1-receptor blockage induces weight loss and reduces the risk to develop the metabolic syndrome with its associated cardiovascular complications. These effects are mediated by central and peripheral pathways. Interestingly weight loss is mainly achieved by a reduction of visceral fat mass. Therefore we investigated fat depot-specific differences on adipocyte differentiation, inflammation, and oxidative metabolism in CB1-receptor knockout cells.
Materials and methods: We used newly generated inguinal and epididymal adipose cell lines from CB1-R knockout mice. Differences in differentiation were measured by fat specific oil red o staining and quantitative RT-PCR-based mRNA expression analysis of key differentiation markers. Induction of apoptosis was evaluated by using a cell death detection ELISA and performing protein analysis of p53 phosphorylation. Inflammation markers were quantified on RNA level. For analyzing the process of transdifferentiation we measured oxygen consumption and mitochondrial biogenesis.
Results: Differentiation was reduced in visceral adipocytes from CB1-receptor knockout mice as compared to wildtype controls. All markers of late differentiation, including AP2, GLUT 4 and PPAR gamma were decreased in these CB1-R-KO cells. Moreover, the inhibitory preadipocyte factor, Pref-1, was elevated. Furthermore, we found a significant induction of apoptosis (increased by 51%) in these cells from the visceral fat depots. In contrast, subcutaneous cells from CB1-R knockout mice showed an accelerated differentiation as well as a reduced induction of apoptosis (decreased by 41%). Inflammation was increased in visceral fat cells, as analyzed by the expression pattern of IL-6 (+357%), MCP-1 (+326%), TNF α (+371%), whereas in subcutanous adipocytes these markers were declined by -60%, -26%, -32% respectively. In addition, subcutaneous CB1-R knockout cells were more sensitive towards a conversion into a brown fat phenotype. UCP-1 expression in these cells was significantly elevated by 176% in preadipocytes and 285% in fully differentiated adipocytes. Moreover, PGC-1 α expression was augmented by 152% and by 140%, respectively. Finally, we found an increase in mitochondrial biogenesis demonstrated by mitochondrial fluorescence staining and RNA expression pattern of TFAM and NRF-1 in these cells. In line with these data, there was also an increase in oxygen consumption by 83% in subcutaneous preadipocytes as well as an 92% enhancement in fully differentiated cells compared to wildtyp controls.
Conclusion: In conclusion, we found depot-specific effects on differentiation, apoptosis, inflammation and oxidative metabolism in CB1-receptor knockout cells. Visceral adipocytes showed a lack of differentiation and an enhancement of apoptosis. In contrast to visceral fat cells, subcutaneous cells expressed an antiinflammatory cytokine profile and were more sensitive towards a conversion into a brown fat cell phenotype. Thus, CB1 receptor-mediated pathways differentially target adipose tissue depots to a dual effect that minimizes cardiometabolic risk, on the one hand, by diminishing fat, and that enhances thermogenesis in subcutaneous adipocytes, on the other.
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Portal IL-6 determines the effect of fat tissue transplantation on glucose homeostasis

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Background and aims: Visceral obesity has been associated with insulin resistance, however the molecular mechanisms relating visceral fat accumulation and hepatic insulin resistance (portal theory) are still not well known. The portal theory implicates increased drainage of pro-inflammatory cytokines and lipids from portal drained adipose tissue directly to the liver. We applied herein a novel adipose tissue transplantation approach to investigate a potential effect of adipose tissue localization and in particular of venous drainage (caval versus portal) on glucose metabolism. Moreover, we hypothesized that IL-6 is a major contributor to hepatic insulin resistance associated with visceral fat accumulation.
Materials and methods: Epididymal fat pads of six weeks old C57Bl6J donor mice were transplanted either to the mesenterium (portal venous drainage) or to the peritoneum (caval venous drainage) of healthy littermates. Sham-operated mice were used as control. After five weeks of transplantation glucose metabolism was assessed by glucose tolerance test (2g/kg body weight) and by hyperinsulinemic-euglycemic clamp.
Results: Mice receiving a portal-drained fat transplant developed impaired glucose tolerance compared to mice receiving a caval-drained transplant ($p< 0.001$) and to sham-operated ($p<0.01$) mice. In portal transplanted mice, glucose infusion rate (GIR) and insulin-mediated inhibition of hepatic glucose production (HGP) during hyperinsulinemic-euglycemic clamp was reduced compared to sham-operated mice ($p< 0.05$) demonstrating the development of hepatic insulin resistance in portal transplanted mice. This was also con-

firmed by reduced insulin-stimulated Akt phosphorylation ($p< 0.01$) in livers of portal transplanted mice. In contrast, hepatic lipid content and Kupffer cell activation was not different. Interestingly, Fas-ligand and interleukin-6 mRNA expression was increased in portal transplanted fat pads. Moreover, IL-6 levels of portal transplanted mice were elevated in portal compared to systemic plasma samples whereas no difference was found in sham-operated mice. Intriguingly, mice receiving a portal drained IL-6-deficient fat transplant showed normal glucose tolerance and hepatic insulin sensitivity. In addition, expression of pro-inflammatory cytokines was significantly reduced in IL-6-deficient transplants.
Conclusion: These results demonstrate an important role for venous drainage of adipose tissue on glucose homeostasis. In addition, IL-6 seems to play a major role in the development of hepatic insulin resistance associated with visceral fat accumulation.
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Hyperactivation of inflammasome-mediated caspase-1: a new mechanism underlying increased inflammatory activity in visceral adipose tissue
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Background and aims: Abdominal adipose tissue, stored viscerally (VAT) or subcutaneously (SAT), is metabolically active and secretes a wide variety of adipokines and cytokines. It has been suggested that VAT possesses more pro-inflammatory properties characterized by an enhanced release of cytokines as compared to SAT. The pro-inflammatory trait of VAT may account for its stronger correlation with insulin resistance, relative to SAT. The inflammatory IL-1 family members IL-1β and IL-18 are increased in obese subjects and negatively affect insulin signaling. The inactive pro-forms of both cytokines are processed into active IL-1β and IL-18 by a cysteine protease called caspase-1. Activation of this enzyme is mediated by the inflammasome, which involves formation of a complex between NOD like receptors and the adapter protein ASC. In this study, we assessed the presence of the inflammasome in human adipose tissue and tested whether inflammasome-dependent caspase-1 activation is more dominant in abdominal VAT compared to SAT.
Materials and methods: Paired abdominal SAT and VAT biopsies were obtained from ten mildly obese subjects (BMI: 25-28 kg/m^2; aged 40-60 years). Intact adipose tissue fragments were immediately cultured for 24 hours from both depots after which medium, RNA, and protein lysates were collected to determine the expression of the inflammasome components and the secretion levels of pro-inflammatory cytokines.
Results: *Ex-vivo* experiments using adipose tissue explants cultures revealed a higher release of bioactive IL-1β (10-fold; $P < 0.05$) and IL-18 (4-fold; $P < 0.05$) from VAT compared to SAT. The increased secretion of both cytokines was significantly reduced when caspase-1 activity was blocked by the specific inhibitor pralnacasan. Although caspase-1 protein was expressed in both adipose tissue depots, western blot analyses and caspase-1 activity assays revealed a 3-fold ($P <0.05$) up-regulation of active caspase-1 protein in VAT in line with the enhanced production of IL-1β and IL-18. In addition to the augmented caspase-1 activity, protein levels of the inflammasome complex members ASC and the Nod like receptor NLRP3 were significantly higher in VAT. Fractioning of VAT into mature adipocytes and stromal vascular cells revealed that caspase-1 gene expression mainly originates from adipocytes, while ASC was found to be more expressed in the stromal vascular cells. Concurrent with the enhanced bioactive IL-1β secretion, IL-6 and IL-8 release was also induced (3-fold; $P < 0.05$ and 4-fold; $P < 0.05$, respectively) in the VAT explants while adiponectin secretion was two times lower ($P < 0.05$).
Conclusion: Our results show that NLRP3 inflammasome components and caspase-1 are present in human abdominal adipose tissue and are highly activated in VAT compared to SAT, resulting in an enhancement of IL-1β and IL-18 secretion. These findings imply that inflammasome-dependent caspase-1 activation contributes to the pro-inflammatory status of VAT.

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The role of TGF-beta/Smad3 signalling in the pathogenesis of obese fat tissue
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Background and aims: Obesity is serious problem because it causes many kind of life-style related illness, such as type2 diabetes, hypertension, and dyslipidemia. In recent years, obesity is recognized to closely associate with inflammation, because hypertrophic visceral adipose tissue secretes a variety of inflammatory cytokines that lead to insulin resistance. TGF-β has a wide range biological effect, differentiation, proliferation, immunomodulation, and so on. We have previously reported the role of TGF-β on atherosclerosis and diabetic nephropathy, using mice lacking Smad3, a major mediator of TGF-β signaling. TGF-β is known to have inhibitory effect for adipocytogenesis, but the detail molecular mechanism is not fully understood. On the other hand, several studies have shown that cross-talk between TGF-β/Smad3 and Wnt-β-catenin signaling, also known to have inhibitory effect for adipocytogenesis, plays important roles in the regulation of cell differentiation. In this study, we aimed to clarify the role of TGF-β/Smad3 signaling in the pathogenesis of obese fat tissue, and investigate the role of cross-talk between TGF-β/Smad3 and Wnt/β-catenin signaling in adipocytogenesis.
Materials and methods: (1) In order to clarify the expression of TGF-β/Smad3 signaling in obese fat tissue, we isolated epididymal white adipose tissue of 12-week old obese *ob/ob* mice and wild type (WT) mice, and analyzed the expression of mRNA and protein related to TGF-β/Smad3 signaling. (2) To clarify whether TGF-β inhibits adipocytogenesis dependent on Smad3, WT and Smad3 KO (KO) mouse embryonic fibroblasts (MEFs) were induced to differentiate into adipocyte with or without 1 ng/ml TGF-β. (3) To clarify the role of cross-talk between TGF-β/Smad3 signaling and Wnt-β-catenin signaling in adipocytogenesis, WT and KO MEFs were stimulated with 1 ng/ml TGF-β, and then evaluated the translocation of β-catenin into nucleus. Moreover, HW preadipocytes were infected with retrovirus carrying empty vector or cby, an antagonist of β-catenin, and then differentiated into adipocytes with or without 1 ng/ml TGF-β. (4) To clarify the role of TGF-β/Smad3 signaling *in vivo*, eight-week-old WT and KO mice were fed high fat diets for 8 weeks. The food intake and body weight were recorded. After 8 weeks, we performed insulin tolerance test and isolated epididymal WAT for histological and genetic analyses.
Results: (1) mRNA and protein expression of TGF-β, and phosphorylation of Smad3 were increased in the epididymal WAT of *ob/ob* mice. (2) TGF-β suppressed adipocyte differentiation on WT MEF, but this inhibitory effect was attenuated on KO MEF. (3) TGF-β promoted translocation of β-catenin into nucleus on WT MEF, but this effect was attenuated on KO MEF. On the other hand, TGF-β suppressed adipocyte differentiation almost completely not only on empty vector infected HW cells but also on cby-infected HW cells. (4) Despite the amount of food intake was similar between two groups, the percent increase in the body weight was significantly larger in KO mice. KO mice were more insulin-sensitive than WT mice. WAT from KO displayed a larger number of adipocytes with a smaller cell diameter.
Conclusion: TGF-β is highly expressed in obese fat tissue, and it inhibits adipocytogenesis via Smad3 and contributes to development of insulin-resistance. TGF-β emphasizes Wnt-β-catenin signaling, but its inhibitory effect on adipocytogenesis may be not dependent on Wnt-β-catenin signaling.

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PI3Kγ in non-hematopoietic cells plays a major role in the promotion of obesity, inflammation and glucose intolerance
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Background and aims: Obesity is associated with a state of chronic low-grade inflammation (metabolic inflammation), which is believed to play an important role in the pathogenesis of obesity and type-2 diabetes. Metabolic inflammation is characterized by leukocyte infiltration into obese adipose tissue, and polarization of T-cells and macrophages toward a more pro-inflammatory cellular population. The lipid kinase phosphoinositide 3-kinase γ (PI3Kγ) has previously been shown to be central in leukocyte chemotaxis

in different models. Furthermore PI3Kγ was shown to play a major role in β-adrenergic receptors signaling within cardiomyocytes, and in angiotensin II signaling in vascular cells. Therefore PI3Kγ is a likely candidate signal transducer at the interface between inflammation and metabolism. In this study we investigated the role of PI3Kγ in diet-induced obesity, metabolic inflammation, and insulin resistance.
Materials and methods: C57BL/6J mice (wt) and mice with a targeted Pi3kγ locus (Pi3kγ-/-) were exposed to a high-fat diet (60% of calories from fat) for 16 weeks. Body weight was measured weekly to determine growth curves. Analysis of body composition, energy balance, in-vivo and ex-vivo calorimetry were performed. To test the role of PI3Kγ in glucose homeostasis and insulin sensitivity we performed glucose tolerance test, insulin tolerance test, and hyperinsulinemic-euglycemic clamp. To learn about the cell-type implicated in the metabolic action of PI3Kγ we have generated mice lacking PI3Kγ either in hematopoietic or non-hematopoietic cells by adoptive transfer. To test the role of the kinase dependent and independent functions of PI3Kγ we have investigated mice expressing a mutated form of PI3Kγ where its kinase function is selectively blocked (Pi3kγKD/KD). Gene expression profiling was performed by DNA microarrays, and by real-time PCR.
Results: When placed on chow diet, wt and Pi3kγ-/- mice display a similar phenotype. However, when fed with high-fat diet Pi3Kγ-/- mice are resistant to diet-induced obesity, mainly because of increased energy expenditure. Insulin and glucose tolerance were markedly improved in Pi3kγ-/- animals versus wt mice, and hyperinsulinemic-euglycemic clamp revealed a four-fold increase in insulin sensitivity in Pi3kγ-/- mice compared to controls. Metabolic inflammation was also markedly decreased in Pi3kγ-/- mice compared to wt mice. Bone marrow transplantation experiments mapped the role of PI3Kγ in diet-induced obesity,-inflammation, and -glucose intolerance in the non-hematopoietic compartment. When placed on high-fat diet Pi3kγKD/KD mice also showed a leaner phenotype, improved glucose homeostasis, and decreased inflammation compared to wt mice.
Conclusion: We demonstrate here for the first time that the PI3Kγ activity in non-hematopoietic cells plays a major role in the promotion of diet-induced obesity, metabolic inflammation, and insulin resistance by a molecular mechanism involving its kinase activity.
Supported by: the Swiss National Science Foundation grant SNF31

OP 37 Type 1 diabetes mellitus: incidence, natural history, morbidity and mortality

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Thirty years of prospective nationwide incidence of childhood type 1 diabetes: the accelerating increase by time tends to level off in Sweden
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Background and aims: A rising incidence in childhood type-1 diabetes (T1D) has been reported from many countries during the last 20 years. Second to Finland, Sweden has the highest reported nationwide incidence of T1D in the world (>40/100.000). Over a 20 year period (1978 - 1997) the incidence of T1D among ages <15 yrs was almost doubled in Sweden, with the largest increase among children aged 0-5 yrs. The change over time has been partly explained by changes in lifestyle causing rapid early growth and weight development. In the present 30 yr follow up (1978 - 2007) of the Swedish Childhood Diabetes Register we describe the current time trend by age, sex and birth cohort, and analyze the changes in incidence using statistical models. We also aim to discuss the possibility to predict numbers of new cases of childhood onset T1D using generalised models over long periods of time. For discussion we also examine possible correlations between time trend in T1D incidence and indicators that mirror calorie-intake.
Materials and methods: The Swedish Childhood Diabetes Registry has recorded cases of childhood onset T1D (0-14yrs) since 1 July 1977 with a high level of coverage (96-99% of cases). This study is based on 14 721 incident cases of childhood onset T1D occurring 1 Jan 1978-31 Dec 2007. Incidence numbers were calculated after extracting population data from Statistics Sweden. Official population projections were used to predict numbers of new cases in Sweden in 2017, 2027 and 2037. Generalized additive models were fitted for Poisson distributions and the impact of calendar year at onset in five years age groups, sex and interaction terms were tested. Model coefficients with significant goodness of fit were used for the prognosis of the trends in incidence. Finally a coefficient of determination was calculated to determine the extent to which incidence of child hood onset T1D and yearly soft drink consumption could be related.
Results: Age and sex specific incidence rates varied from 21·6 (95 % CI 19·4 - 23·9) during 1978-1980 to 43·9 (95 % CI 40·7 - 47·3) during 2005-2007. Cumulative incidence shifted to younger age at onset over the first 22 years, but from the birth year 2000 a reversed trend was seen. Using a model including all calendar years, age and sex the predicted number of incident cases may more than triple the next 30 years period (648 in 2007 and 2294 in 2037). An ecological analysis using soft drink consumption as a marker for eating habits and high energy intake showed a strong correlation with incidence rate in Sweden (R^2= 0·84).
Conclusions: Childhood T1D increased dramatically and shifted to younger age at onset the first 22 years of the study period, but since year 2000 for the first time we report a reversed trend. The predicted almost tripling of incident cases over the next 30 years is thus probably an overestimation. No distinct conclusions can be made from the ecological analysis, but the strong correlation between increasing soft drink consumption and increasing trend of T1D add an interesting contribution to the discussion.
Supported by: VR and VLL

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The changing natural history of type 1 diabetes: contrasts between those diagnosed in the 1960s and 1970s
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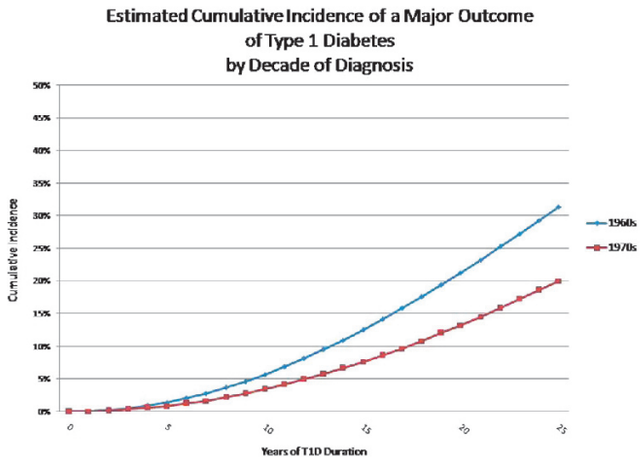
Background and aims: Individuals with type 1 diabetes (T1D) face a number of different complications, or outcomes, and thus it is important to view, from a management perspective, a broad combination of major outcomes of diabetes (MOD). Little is known concerning the cumulative risk of MOD for childhood onset T1D and to what extent this may have declined with improved care.
Materials and methods: To examine these changes, data from the 22 year historical prospective Pittsburgh Epidemiology of Diabetes Complications study of childhood onset (<17 years) T1D diabetes were used. Participants,

diagnosed between 1960 and 1980 at Children's Hospital of Pittsburgh, were grouped by year of diabetes diagnosis (1960-1969, n=321 and 1970-1980, n=368). MOD was defined as diabetes-related death, CAD (MI, revascularization), stroke, end-stage renal disease, amputation or blindness. Cumulative incidence estimates (figure) were calculated using Weibull accelerated failure-time modeling.
Results: By 25 years of diabetes duration, 34% (110) of individuals from the 1960s cohort had experienced such events (5% had died, 6% had CAD, 2% stroke,12% ESRD, 8% blindness, 1% amputation) while for the 1970s cohort only 18% had suffered a MOD (3% had died, 2% had CAD, <1% stroke, 2% ESRD, 9% blindness, 2% amputation). These observed cumulative incidences were close to those predicted using Weibull models, which estimated that 31% (95% CI 27.4, 35.8) of the 1960s cohort and 20% (95% CI 16.7, 23.9) of the 1970s cohort would have an MOD event by 25 years of T1D duration (p=0.0001). The majority of the care for the 1960s cohort was before the advent of self-monitoring of blood glucose and HbA1c testing, while the reverse is true of the 1970s cohort.
Conclusion: While these results suggest encouraging falls in many components of MOD, it is striking that no reduction is seen for blindness or amputation, which together now account for 60% of MOD events in the first 25 years of T1D in the 1970s cohort as opposed to only 27% in the 1960s. Further attention should be paid to the total morbidity burden of those with T1D and to understanding why blindness and amputation are not being delayed or prevented.



Supported by: NIH

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HbA_{1c} levels and hospital admission in people with type 1 diabetes
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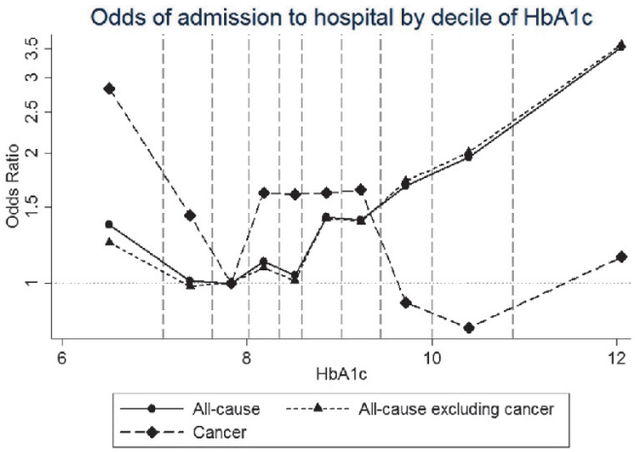
Background and aims: There has been recent concern that very tight glycaemic control might be associated with an increase in morbidity in people with diabetes. We assessed the relationship between deciles of HbA1c and hospital admissions in patients with type 1 diabetes.
Materials and methods: The Scottish Care Information - Diabetes Collaboration (SCI-DC) is a dynamic national register of diagnosed cases of diabetes in Scotland. These data were linked to centralised data on hospital admissions from Information Services Division (ISD) of NHS National Services. We identified 24,760 people with type 1 diabetes during January 2005 to December 2007 and include 19,777 patients with complete recording of covariates. Patients were divided into deciles according to levels of HbA1c. All-cause admission to hospital was the primary outcome. Logistic regression models were used to estimate the association between HbA1c and all cause admissions expressed with decile 3 (mean HbA1c 7.8%, range 7.6%-8.0%) as referent and adjusted for potential confounding factors including age, sex, previous vascular disease, creatinine, body mass index and diabetes duration.
Results: 8.1 % of people had HbA1c <7.0% and 16.2% under 7.5%. There was a J-shaped relationship of HbA1c to all hospital admissions with highest likelihood of admission (adjusted odds ratio 3.54, 95%CI 3.04-4.12) in the highest HbA1c decile (12.1%; 10.8-18.4%) but also increased admissions (adjusted OR 1.36, 95%CI 1.13-1.64) in the lowest HbA1c decile (6.5%; 4.4-7.1%). Cancer admissions showed a broadly inverse relationship with HbA1c (adjusted OR 2.38, 95%CI 1.33-6.03) in the lowest decile of HbA1c, see Figure. Vascular admissions showed a positive relationship with HbA1c with significantly higher likelihood of admission in HbA1c deciles 7 through 10 (9.03-18.4%). Likelihood of vascular admission was not significantly increased in the lowest decile of HbA1c (adjusted OR 1.10, 95%CI 0.65-1.87) and an increase in all cause admissions remained even after excluding admissions due to cancer and hypoglycaemia (adjusted OR 1.26, 95%CI 1.07-1.49).
Conclusion: Low and high mean HbA1c values were associated with increased admission to hospital with lowest rates of admission for any cause in deciles 2 through 5 (HbA1c 7.1-8.7%) People with the lowest levels of HbA1c had an increase in cancer admissions and this likely reflects reverse causality in this observational dataset. However, an increase in admissions remains even after exclusion of cancer and hypoglycaemic admissions. Overall the likelihood of admissions increases markedly with HbA1c and the highest levels of HbA1c marks out a group with high likelihood of admission and attendant hospital costs.



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Time trends of mortality in patients diagnosed with type 1 diabetes below 30 years between 1970-1999 in Finland
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Background and aims: Despite of great advances in the diabetes care, type 1 diabetes (T1D) is still associated with a premature mortality due to both acute and long-term diabetic complications. The aim of this study was to assess long-term time trends in mortality among patients diagnosed with early-onset (0-14 years) and late-onset (15-29 years) T1D in Finland. In addition, we aimed to study causes of deaths addressing the changes in the mortality over time.
Materials and methods: Individuals diagnosed with T1D during 1970-1999 (n=17,306) were identified from the Finnish nationwide population-based registers. Vital status and causes of deaths were obtained from the Finnish Cause of Death Register until the end of 2007. Patients were stratified into subcohorts by the year of diagnosis: 1970-74, 1975-79, 1980-84, 1985-89, 1990-94 and 1995-99. Cumulative mortalities were evaluated using Kaplan-Meier method. Crude mortality (per 100,000 person-years) and standardized mortality rates (SMR) were calculated. Time trend evaluation in the SMRs was performed by means of Poisson regression modeling.
Results: A total of 1,338 deaths were observed during 370,733 person-years of follow-up giving an all-cause mortality rate of 361 (342-382). The crude mortality was higher in the late-onset than in the early-onset cohort; 531 vs 245. However, the SMR was similar, 2.9 (2.6-3.1) in the early-onset and 2.7 (2.6-2.9) in the late-onset cohort. Women had higher SMR in both cohorts, 3.8 (3.4-4.1) and 3.5 (3.0-4.0), compared to men, 2.5 (2.3-2.6) and 2.4 (2.2-4.7). Overall cumulative mortality at 35 years of duration of diabetes was 17.9% (17.0-18.8). There was no beneficial development in the long-term prognosis by diagnosis years. However, a decreasing trend was seen in the 20-year cumulative mortality in the early-onset cohort from 4.7% (3.7-5.8) to 4.3% (3.3-5.2), 3.6% (2.8-4.5) and 2.7% (1.9-3.4) in the subcohorts 1970-74, 1975-79, 1980-84 and 1985-89. The SMR at 20 years duration of diabetes decreased

from 2.6 (2.0-3.2) to 1.5 (0.8-2.2) from 1970-74 subcohort to 1985-89 (p for trend 0.07). There was, however, an inverse trend in the late-onset cohort with corresponding rates 4.4% (3.3-5.6), 5.9% (4.6-7.1), 6.5% (5.1-7.8) and 7.9% (6.4-9.4). The SMR increased from 1.3 (1.0-1.7) to 2.7 (2.0-3.3) from 1970-74 to 1985-89 (p for trend 0<0.001). At 20-years of duration of diabetes chronic diabetic complications explained 38% of the deaths in the 1970-74 subcohort decreasing to 17% in the 1985-89 subcohort among those with early-onset diabetes. In the late-onset cohort mortality decreased from 58% to 26%. The proportion of alcohol and drug related deaths was high in the early-onset diabetes, appr. 25%, while it increased to 39% in the late-onset cohort. SMR for alcohol-related deaths was 4.4 (3.1-5.7) and 2.7 (2.2-3.3) in the early and late-onset cohorts and was equal for men and women. SMR for suicides was significant only in women with early-onset diabetes, 3.3 (1.9-4.7).
Conclusion: We found improvement in survival of people with early-onset T1D. On the contrary, individuals with late- onset T1D showed worse survival after the 1980s compared to the 1970s. Reduction in chronic diabetic complications was, however, replaced by alcohol- and drug-related mortality.

OP 38 Diabetic nephropathy - clinical trials

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Intensive glucose control is renoprotective in type 2 diabetes: New analyses from ADVANCE

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Background: Blood glucose is an important determinant of kidney disease among patients with type 2 diabetes. In these analyses we determine the effects of intensive glucose lowering, targeting an HbA1c of less than or equal to 6.5%, a level below the currently recommended targets, on a range of renal outcomes among 11,140 patients who had type 2 diabetes and participated in the Action in Diabetes and Vascular disease: preterAx and diamicroN-MR Controlled Evaluation (ADVANCE) study.
Methods: Patients were randomly assigned to intensive glucose control based on the use of gliclazide MR and other therapy as required to achieve an HbA1c level less than or equal to 6.5%, or standard glucose control. Treatment effects on total renal events, new or worsening nephropathy, new-onset microalbuminuria (UACR 30 to 300 g/mg), new-onset macroalbuminuria (UACR >300 g/mg), progression of albuminuria by at least 1 stage (from normoalbuminuria to either micro- or macroalbuminuria or from micro- to macroalbuminuria) and regression of albuminuria by at least 1 stage, were assessed.
Results: After a median follow-up of 5.0 years, the mean HbA1c level achieved in the intensive control group was 6.5% as compared with 7.3% in the standard control group. As compared to standard glucose control, intensive glucose control reduced the risk for total renal events by 11% (95% CI =(5%-17%), *P*<0.001), new or worsening nephropathy by 21% (95% CI, 7-34%,p=0.006), new-onset microalbuminuria by 9% (95% CI, 2-5%, p=0.018), new-onset macroalbuminuria by 30% (95% CI, 15-43%, P<0.001) and progression of albuminuria by 10% (95% CI, 2-16%, p=0.028). In patients with albuminuria at baseline, regression by at least one stage occurred in 62% of intensively treated patients, with the majority achieving normoalbuminuria. Compared to standard glucose control, intensive glucose control increased regression of albuminuria by 15% (95% CI, 5-26%, P<0.002). Effects of active treatment on total renal events were consistent across subgroups defined by median HbA1c level at baseline (p for interaction>0.1).
Conclusion: The gliclazide MR-based intensive glucose control regimen, aiming for an HbA1c level less than or equal to 6.5% in patients with established type 2 diabetes, provided renal benefits including regression or normalisation of albuminuria. This renoprotection, is evident even among those with initial HbA1c levels < 7% and we could not identify an HbA1c threshold below which renal benefit was lost.
Supported by: Servier and NHMRC of Australia

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Prevention of microalbuminuria in type 2 diabetes (ROADMAP Trial)

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Background and aims: Microalbuminuria is an early sign of diabetic nephropathy and increased cardiovascular risk. We investigated whether early treatment with an angiotensin receptor blocker (ARB) in diabetic subjects with normal albumin excretion delays the occurrence of microalbuminuria and concomitantly recorded cardiovascular and renal events.
Materials and methods: We studied 4,447 patients with type 2 diabetes and at least one additional cardiovascular risk factor in a randomized, double-blind, multicentre, controlled, and event-driven (onset of microalbuminuria) trial. They received either 40 mg olmesartan or placebo once daily for a median duration of 3.2 years. In both groups, additional antihypertensive drug treatment (except ACE inhibitors or ARBs) was used to reach the target blood pressure of <130/80 mmHg.

Results: Baseline eGFR, blood pressure and cardiovascular disease (CVD) risk profiles were comparable in both groups. Nearly 80% of the subjects in the olmesartan group and 71% in the placebo group achieved target blood pressure at month 48. Kaplan-Meier analysis showed a cumulative incidence of microalbuminuria of 8.2% (n=178) with olmesartan and 9.8% (n=210) with placebo which represents a risk reduction of 23% (HR: 0.77; 95.1% CI: 0.63 to 0.94; p= 0.01) in favour of subjects receiving olmesartan. At study end eGFR was lower in the olmesartan-treated subjects (80.1 vs. 83.7 mL/min/1.73 m^2, p<0.001). In both groups 23 subjects had a doubling of the baseline serum creatinine. Overall cardiovascular morbidity and mortality rate was low and similar between groups with cardiovascular morbidity events in 81 (3.6%) and 91 (4.1%) patients, and total mortality in 26 (1.2%) and 15 (1.7%) on olmesartan and placebo, respectively (p> 0.1). Cardiovascular mortality however was higher (15 (0.7%) vs. 3 (0.1%); p= 0.01) in the olmesartan group, possibly due to hypotensive episodes in subjects with pre-existing CVD.
Conclusion: In subjects with type 2 diabetes and excellent blood pressure control early treatment with the ARB olmesartan showed a significant risk reduction regarding the 'time to onset of microalbuminuria'. ClinicalTrials.gov ID no.: NCT00185159.
Supported by: Daiichi Sankyo

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Renin angiotensin system blockade is effective in preventing microalbuminuria in hypertensive but not normotensive people with type 2 diabetes; further analysis of the DIRECT Programme
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Background and aims: Renin angiotensin system (RAS) blockade prevents microalbuminuria in people with type 2 diabetes (T2DM) at high cardiovascular risk. However, most of these studies measured albumin:creatinine ratio on a single spot urine to define microalbuminuria. Using multiple timed overnight collections the DIRECT Programme could not demonstrate a benefit of RAS blockade on the development of persistent (3 of 4 consecutive samples > 20 μg/min) microalbuminuria. We re-analysed our data using the less stringent microalbuminuria definition of a single value >20μg/min to be more consistent with previous studies.
Materials and methods: 1905 people with T2DM and mild/moderate retinopathy were randomised to Candesartan (titrated to 32mg/d) or placebo. At baseline all were normoalbuminuric (median albuminuria 5.5 (IQR 3.5,8.5) μg/min. 62% were hypertensive (mean BP at entry 139/79 mmHg) and 38% normotensive (BP < 130/85, mean 123/75 mmHg). Subjects collected 2 timed overnight urine collections annually for at least 4 years.
Results: The adjusted risk (Hazard Ratio - HR) and (95% CI) for microalbuminuria for Candesartan versus placebo was 0.80 (0.67,0.96) p = 0.016. This beneficial effect was similar in normotensive (HR 0.81 (0.61,1.09) p = 0.166) and hypertensive (0.79 (0.63,0.99) p = 0.037) individuals at baseline although remaining statistically significant only in the latter. In contrast, no beneficial effect of Candesartan was seen using our more stringent definition of persistent microalbuminuria (HR 0.80(0.58,1.11); 0.66(0.40,1.09) ; and 0.91 (0.60,1.40) for the entire group, normotensive and hypertensive subjects respectively; p = NS for all.
Conclusion: Candesartan is effective at preventing microalbuminuria in people with T2DM and hypertension using the looser definition of a single positive sample. No effect was seen in people with T2DM and normal blood pressure. These results highlight the need for careful and standardized definitions of early nephropathy in intervention trials.
Supported by: Astra Zeneca and Takeda

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Aldosterone reduction during 24 weeks of treatment with aliskiren or placebo added to losartan in patients with type 2 diabetes and nephropathy, an AVOID substudy
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Background and aims: Aldosterone suppression reduces albuminuria in diabetic and non-diabetic patients, and is known to improve cardiorenal prognosis. This study assessed the effects on urinary aldosterone, plasma renin activity (PRA) and plasma renin concentration (PRC) of direct renin inhibition with aliskiren (ALI) in combination with the ARB losartan (LOS) and optimal antihypertensive therapy in the Aliskiren in the EValuation of PrOteinuria In Diabetes (AVOID) study.
Materials and methods: In the AVOID study, 599 patients aged 18-85 years with hypertension and diabetic nephropathy received 6 months' ALI (150 mg force titrated to 300 mg after 3 months) or PBO added to LOS 100 mg and optimal antihypertensive therapy. Study exclusion criteria comprised non-diabetic kidney disease, urinary albumin:creatinine ratio (UACR) >3500 mg/g, eGFR <30 ml/min/1.73 m^2 and serum potassium >5.1 mmol/l. Urinary aldosterone, PRA and PRC were measured at baseline of the double-blind period and after 24 weeks in a subset of 133 patients.
Results: ALI added to LOS provided large reductions from baseline in urinary aldosterone compared with adding PBO (−24% vs. −4%, p=0.017) at week 24. There was no significant difference between the aliskiren and placebo groups in the proportion of patients with aldosterone breakthrough (ALI 35%, PBO 46%, p=0.199). There was no correlation between change in urinary aldosterone levels and change in UACR or change in systolic blood pressure (SBP). ALI treatment reduced PRA by 90% at 24 weeks (p<0.001); PBO treatment had no significant effect. PRC increased by 228% from baseline with ALI and decreased by 17% with PBO (p<0.001).
Conclusion: Adding ALI to LOS and optimal antihypertensive therapy provided significant, long-term reductions in urinary aldosterone beyond those provided by ARB and optimal antihypertensive therapy. Reduction in PRA by ALI treatment may be a potential mechanism behind the reduction in urinary aldosterone levels.
Supported by: Novartis

OP 39 CNS, appetite control and cognition

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Acute administration of the GLP-1 receptor agonist, Exenatide, restores impaired central responses to food ingestion in type 2 diabetes

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Background and aims: An appetite control system that allowed continued eating when food is available would be of evolutionary advantage in a hunter-gatherer lifestyle but would predispose to obesity in the modern world. Incretins, such as GLP-1, are secreted by the gastrointestinal tract in response to food ingestion and are involved in the satiety response that terminates eating. Incretin responses to food ingestion are abnormal in obesity and Type 2 diabetes (T2DM) and incretin-based therapies can deliver weight loss and improved diabetic control. The aim of our study was to use the GLP-1 receptor agonist Exenatide to explore the central control of appetite and satiety in people with T2DM, using functional magnetic resonance imaging (fMRI) and the food cue of food image viewing.
Materials and methods: 12 subjects with lifestyle ± metformin treated diabetes (age 55.0 ± 1.8 yrs, BMI 31 ± 1.7, HbA1c 7.1 ± 0.2%) underwent 4 fMRI brain scans, while observing images of food and non-food shown in a block design paradigm, after overnight fast. Satiety and hunger were assessed by visual analogue scales before and after both ingestion and image viewing. On two occasions, subjects consumed a mixed meal (554kcals) as 200 mls chocolate ice cream (FED studies); on two they took 50 mls water to mimic the physical actions of ingestion (FASTED), prior to image viewing. A single subcutaneous injection of either an active GLP-1 receptor agonist (10 mcg Exenatide), or placebo was given immediately prior to each scan. Scans were done in random order with subject and investigator blinded to the nature of the injection. Changes in blood oxygenation level dependent (BOLD) signal collected during image viewing were analysed with XBAM software. Brain regions with changes in activation in response to food image viewing, shown by changes in BOLD signal, were compared using sum of squares (SSQ). Data from 12 healthy volunteers, age 25± 1.2 yrs, BMI 22±0.8 were available for comparison.
Results: Subjects felt more hungry after water in the fasted state (p=0.01), an effect reduced by Exenatide (p<0.01). Visual cortical activation occurred in all subjects and conditions, with BOLD signal change in response to food and non-food images (p=0.9). In contrast, distinctive BOLD signal changes showed activation in the ventral tegmental area (VTA) in T2DM patients observing food images when FED, which were not present FASTED (p=0.036). The activation was reduced in FED by Exenatide (p<0.02 vs FASTED placebo), to a degree not different from the FED non-diabetic data (p=0.70). The T2DM FED response to food image viewing was not different from the non-diabetic FASTED state (p=0.13).
Conclusion: The VTA is central to perception of the reward value of food and in animal studies its responses are affected by insulin. Its activation pattern in T2DM after eating is different from in health, resembling the healthy response to food cues in the fasted state. Acute elevation of incretin action, by Exenatide, restores the reward circuitry response of the early T2DM in the fed state towards normal. The T2DM activation pattern indicates a failure of satiety and would be expected to encourage prolonged eating. Such a mechanism may contribute to obesity in people with insulin resistant diabetes.
Supported by: Eli Lilly UK

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Abnormal reward processing towards appetizing food in obesity

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Background and aims: Comparative studies have identified an interconnected network comprising of subcortical and frontocortical regions in various aspects of reward processing. This reward circuit plays a key role in guiding appetitive behaviours, and its dysfunctions have been associated with obesity. The aim was to study brain metabolism of glucose and the processing of anticipatory food reward in obesity.
Materials and methods: The brains of 19 morbidly obese (age 46±10 years, BMI 43.9±3.7 kg/m^2) and 16 lean individuals (age 48±10 years, BMI 24.1±2.1 kg/m^2) were studied with 2-[^{18}F]fluoro-2-deoxyglucose positron emission tomography (PET) during euglycemic hyperinsulinemia, and with functional magnetic resonance imaging (fMRI) while anticipatory food reward was induced by repeated presentations of appetizing and bland food pictures.
Results: We found that in obese individuals glucose metabolic rate (GMR) of the caudate nucleus was elevated when compared to controls (4, 8, 4, T = 3.97, p = .03 SVC), but not in any other a priori region of interest. Responses to all foods (appetizing and bland) were higher in obese patients than in controls in the left inferior occipital gyrus (-39, 59, -8, T = 3.76, p < .005, unc.), left amygdala (-30, -10, -27, T = 3.89, p < .005, unc.), right posterior cingulate cortex (8, -38, 19, T = 3.84, p < .005, unc.), and right postcentral gyrus (56, -16, 30, T = 3.81, p < .005, unc.). However, responses were lower in obese than in lean subjects in the left superior frontal gyrus (-24, 49, 4, T = 3.95, p < .005, unc.). Obese patients showed elevated functional responses to appetizing vs. bland food viewing compared with controls specifically in the right caudate nucleus, whereas they had lowered functional responses in the left insula, lateral frontal cortex, superior parietal lobule, right orbitofrontal cortex and superior temporal gyrus. The evaluation of functional connectivity of the caudate nucleus revealed that obese patients showed significantly larger coupling between right caudate nucleus and right basolateral amygdala (33 -5 -16, T = 3.92, p < .005, unc.), primary somatosensory cortex (39, -13, 32, T = 3.63, p < .005) and posterior insula (30, 14, 18, T = 3.47, p < .005) than lean control subjects. This abnormal connectivity was specific to obesity, since no task-dependent changes in connectivity while viewing appetizing vs. bland foods were observed in the whole study group.
Conclusion: We found that obese individuals had increased hemodynamic responses in the caudate while viewing appetizing vs. bland foods, and increased amygdala responses to both appetizing and bland foods. Moreover, while viewing appetizing vs. bland foods the functional connectivity of the caudate nucleus and amygdala was increased in the obese vs. lean individuals. Conversely, insular cortex showed elevated responses to appetizing vs. bland foods in lean vs. obese individuals. These data show that adiposity is associated with caudate nucleus's elevated baseline activity, responses to appetizing foods as well as functional connectivity with amygdala and insula while viewing appetizing and bland foods. The elevated amygdala responses to foods and increased amygdalo-striatal connectivity and high tonic GMR in obese patients could be the critical mechanism which would explain overeating in obesity.
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MRI-measured cerebral microbleeds and their relation to cognitive functioning and cerebral activity in patients with longstanding type 1 diabetes mellitus

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Background and aims: Only recently an increased occurrence of cerebral microbleeds (CMB), a magnetic resonance imaging (MRI) marker of vascular fragility, was reported in type 2 diabetic patients, in particular in those with proliferative diabetic retinopathy (DRP), relative to non-diabetic subjects. Here, we explored the relationship of CMBs, cognitive functions and functional brain connectivity, using MRI, neuropsychological assessment and magnetoencephalography (MEG), respectively, in a sample of patients with type 1 diabetes (T1DM) with and without microangiopathy and controls.
Materials and methods: Forty-eight T1DM patients with microangiopathy, 43 T1DM patients without microangiopathy and 42 healthy controls underwent MRI imaging using susceptibility-weighted imaging (SWI) to detect CMBs and neuropsychological assessment to measure cognitive performance. Blood was drawn and cortical communication was recorded by MEG.
Results: Nineteen participants (14.3%) showed one or more CMBs on SWI, of whom 11 had T1DM with microangiopathy (9.0%), 3 were T1DM patients without microangiopathy (2.3%) and 4 were controls (3.0%). Those with CMBs were older (45.8 vs 39.3 P = 0.007). CMBs were mainly located in the

temporal and frontal areas. There were no statistically significant more CBMs in the whole diabetes group compared to controls (P > 0.05), however, a significant linear trend across the 3 groups was observed (P = 0.035). Adjustment for age, gender, hypertension and depressive symptoms yielded significantly more CMBs in T1DM participants with microangiopathy as compared to T1DM patients without microangiopathy and healthy controls (both P > 0.05). Adjusting for either diabetes duration or diabetes age-of-onset, did not change the results. Interestingly, individuals with CMBs did not show cognitive impairments (P > 0.05), but demonstrated lower cortical communication in the lower alpha-band (8 - 10 Hz) compared to all individuals without CMBs (P < 0.05). Per group analyses yielded the same pattern of changes in cerebral communication, albeit in different frequency bands, with preserved cognitive functions. In T1DM patients the presence of CMBs was significantly correlated with later-onset of diabetes (P < 0.05).
Conclusion: Taking into account the relatively small number of patients affected, the results indicate that CMBs are more prevalent in T1DM patients with microangiopathy and are related with changes in cerebral communication. CMB presence was not associated with impaired cognitive functioning, but related to older age at diabetes onset. We hypothesize that the observed changes in cortical communication may reflect a restructuring of neural networks in order to compensate for structural damage, thus allowing cognitive functions to be temporarily spared. Whether CMBs are a marker of future cognitive decline, and whether these abnormalities are diabetes-specific, remains to be determined in large-scaled longitudinal studies.
Supported by: Dutch Diabetes Research Foundation

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Comparison of neuropsychological testing and 99mTc-HMPAO brain SPET findings in patients with diabetes mellitus type 1 and type 2
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Background and aims: Diabetes mellitus is a chronic metabolic disease characterised by macrovascular and microvascular complication. Although the evidence of cognitive deterioration in diabetic individuals is well known, the neuroanatomical substrate of subscribed changes remains uncertain. Evaluate and localise the resting alterations in brain microcirculation and compare them with affected cognitive domains.
Materials and methods: We examined 47 patients, 20 individuals with T1DM (13 men, 7 women), average age 37 ± 12,7 years, 27 individuals with T2DM (14 women, 13 men), average age 60 ± 9,2 years. Diabetic patients were compared with control group composed of 40 non-diabetic age-related individuals. Written consent was obtained from all patients prior the study. Patients performed battery of neuropsychological testing including 9 tasks covering 5 cognitive domains. Individuals underwent 99mTc-HMPAO brain SPET. Vascular and metabolic determinants were recorded. Collected data were analysed using nonparametric statistic methods.
Results: Compared to control group we found in type 1 diabetics changes in working memory, mental flexibility, mental recording, vigilance and information processing (p = 0,01) without significant correlation to gender, age, metabolic compensation or duration of diabetes (p > 0,05). In type 2 diabetes we discovered deterioration of short time memory, mental flexibility, information processing (p < 0,01) correlated with duration of diabetic disease more than 10 years (p = 0,01). Brain SPET revealed hypoperfusion on microcirculatory level in 65 % of T1DM group (thalamus and basal ganglia 30 %, temporal lobe 20 %, parietal lobe 40 %, cerebellum 25 %, occipital lobe 10 %, frontal lobe 10 %). In T2DM cohort was hypoperfusion in 81 % patients (parietal lobe 64 %, temporal lobe 36 %, occipital lobe 27 %, cerebellum 18 %, thalamus and basal ganglia 9 %. In frontal lobe we have seen both hypoperfusion and hyperperfusion in 32%) related to age and duration of diabetes. Comparing the results of neuropsychological testing to brain SPET findings in both diabetic groups we found anatomically equivalent correlation only in verbal memory test, digit span test backwards and Stroop test C.
Conclusion: We confirmed expected cognitive changes in diabetic individuals, which did not correlate with brain SPET findings in all cognitive domains. Therefore we suggest multidimensional patomechanism including changes in perfusion, neurotransmitters and neuronal metabolism.

OP 40 The diabetic patient in the hospital

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Mean glucose during ICU admission is related to mortality by a U-shaped curve; implications for clinical care
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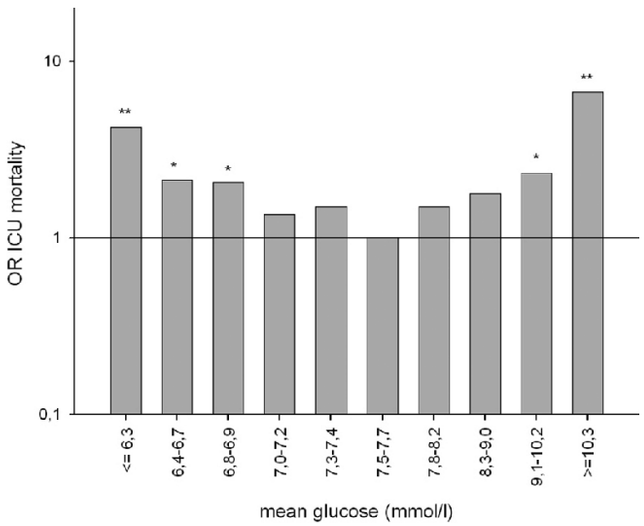
Background and aims: Reducing hyperglycaemia at the ICU reduces mortality but recently the optimal glucose target range has become unclear. We investigated in which way glucose regulation, defined as mean achieved glucose concentration during admission, is associated with ICU mortality, thereby trying to reconcile the conflicting data from the Leuven and NICE-SUGAR trials.
Materials and methods: We performed a retrospective database cohort study including patients admitted to a 20-bed medical/surgical ICU in a teaching hospital between January 2004 and December 2007. 5983 patients were eligible for analysis after excluding readmissions, patients with a withholding care policy and patients with only one glucose value measured. From this population we randomly selected 2435 patients with a surgical/medical ICU admission ratio of 55/45%, to enable comparison with the Leuven and NICE-SUGAR populations. All patients were treated for hyperglycaemia using a fully computerized tight glucose algorithm targeting for glucose values between 4.0 and 7.0 mmol/l. The cohort was subdivided in deciles and logistic regression analysis was performed adjusted for age, sex, severity of disease and admission duration to assess the odds ratio of ICU mortality per glucose stratum.
Results: A median (IQR) of 12 (8-14) glucose values per admission day per patient was collected. The total population and the random sample were comparable regarding all baseline characteristics. We observed a U-shaped relation between mean glucose and mortality, with high mortality in the lowest and highest glucose-stratum, 21.3% and 27.6% respectively. Mean glucose values <7.0 mmol/l and >9.0 mmol/l were associated with significantly increased ICU mortality compared with the stratum with the lowest mortality (OR 2.06-4.24 and 2.33-6.70 respectively; Figure). Limitations of the study were its retrospective design and possible incomplete correction for severity of disease.
Conclusion: Mean glucose during ICU admission is related to mortality by a U-shaped curve. A 'safe range' of mean glucose regulation might be defined between 7.0 and 9.0 mmol/l. The U-shaped curve may help to explain the increased mortality in the intensively treated group of the NICE-SUGAR study but not the low mortality in the intensively treated groups of the Leuven studies. According to these findings and awaiting further studies we recommend treating hyperglycaemia at the ICU in a moderately intensive way, targeting for mean glucose values between 7.0 and 9.0 mmol/l and avoiding hypoglycaemia.



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Accuracy and reliability of continuous glucose monitoring at the ICU; a head to head comparison of two subcutaneous glucose sensors in cardiac surgery patients

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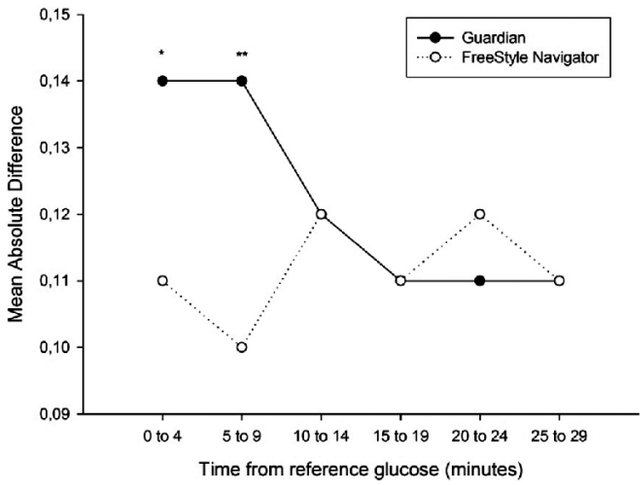
Background and aims: Both hyperglycaemia and hypoglycaemia are common during intensive care unit (ICU) stay and are associated with increased mortality. Continuous glucose monitoring (CGM) is a promising tool to assist glucose control but the accuracy and reliability of these devices in critically ill patients is uncertain. We studied two different CGM devices post-operatively in cardiac surgery patients in an investigator initiated trial.
Materials and methods: Two CGM devices (Guardian RT, Medtronic Minimed; FreeStyle Navigator, Abbott Diabetes Care) were placed subcutaneously in the abdominal area in 60 patients before surgery. Both devices were calibrated simultaneously upon arrival at the ICU after surgery. Further calibrations were performed according to manufacturers' instructions. An arterial blood glucose value was measured with an AccuChek device as a reference value every two hours. Mean absolute difference (MAD) between reference and sensor glucose values was calculated in six 5 minute intervals after the time of the reference glucose, to assess a possible delay for the CGM devices.
Results: In total, 1017 reference glucose values were measured. Of those, 77.8% could be paired with a Guardian and 91.8% with a Navigator glucose value. Missing values indicate technical problems with the device. Median (IQR) MAD was significantly smaller for Navigator compared to Guardian glucose measurements at the first and second interval (0.11 [0.08-0.16] and 0.10 [0.08-0.16] compared to 0.14 [0.11-0.18] and 0.14 [0.11-0.17], p=0.05 and p=0.001, Wilcoxon signed ranks test; figure). The lowest MAD of the Navigator was observed in the second interval, 5-9 minutes after reference glucose. The MAD of the Guardian was lowest after 15-19 minutes. Only for the Guardian there was a significant decrease over time indicating a delay (p=0.01, repeated measures ANOVA). For glucose values ≤6 mmol/l median (IQR) MAD was lower for the Navigator in all intervals, however not significantly (interval 1: Navigator 0.13 [0.10-0.33], Guardian 0.26 [0.17-0.41], p=0.24). The limited number of values ≤6 mmol/l (n=121) could have limited the power of this sub-analysis.
Conclusion: We report that the FreeStyle Navigator CGM system performed better than the Guardian RT in accuracy as well as reliability in post-operative cardiac surgery patients during ICU stay. Remarkably, the MAD of both sensors was quite good as compared to reported data for outpatients. Based on our results we conclude that this device can be used in this group of ICU patients characterized by relatively low disease severity scores and low mortality rates. Whether or not the use of CGM improves glycemic control and mortality has needs further research.



Supported by: an EFSD/LifeScan grant

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Intensive care unit (ICU) glucose monitoring measured in plasma using mid-infrared spectroscopy

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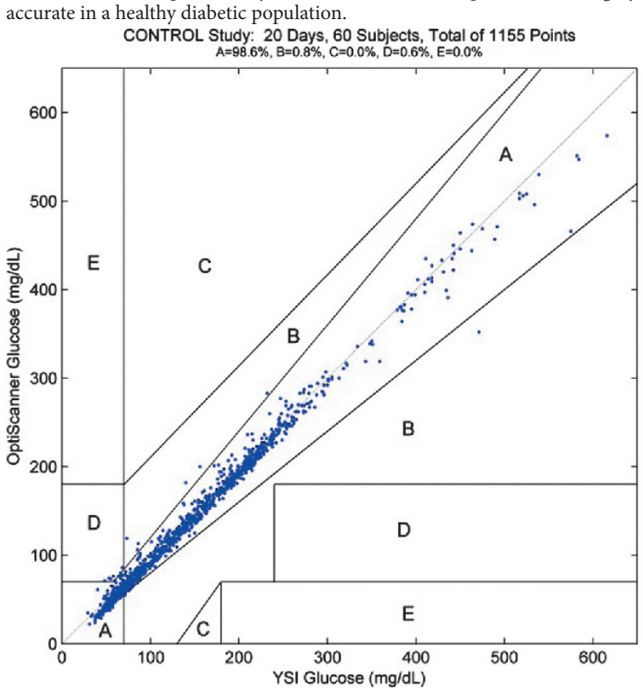
Background and aims: There are increasing calls for a highly accurate, automated system to enable tight glycemic control and avoid hypoglycemia in an ICU setting. The OptiScanner (model 5000) is a glucose monitor based on mid-infrared spectroscopy that draws blood samples (120 µl) and measures plasma glucose concentration approximately every 15 min. The goal of this study was to validate the performance of the OptiScanner at different glycemic levels in a healthy diabetic patient population.
Materials and methods: Sixty people (50 males, age 49 (18-65) years, BMI 29.7 (21.4-40.1 kg/m^2)) with type 1 (n=18) or type 2 (n=42) diabetes were connected to an OptiScanner. Their blood glucose concentrations were kept in a euglycemic (75-180 mg/dl), hypoglycemic (180 mg/dl) range by iv administrations of insulin and glucose. Each OptiScanner blood sample was automatically withdrawn from a forearm vein. Blood samples for reference measurements using the YSI 2300 were withdrawn from the same arm within 60 seconds of the OptiScanner draw and analyzed immediately.
Results: The aggregate data points (1155 paired readings between the OptiScanner and the YSI 2300) were within ISO standard, with 98.6% of the glucose values within +/-20% above 75 mg/dL and +/- 15 mg/dl below this value. A Clark Error Grid analysis showed a total of 1139 points (98.6%) in Zone A. Points outside of A exceeded the A zone boundary by an average of 4.3% and a maximum of 26.4%. The total coefficient for variance was 6.4%. The total r^2 was 0.99.
Conclusion: These preliminary results show that the OptiScanner is highly accurate in a healthy diabetic population.



Supported by: Optiscan Biomedical Corporation

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Randomised study of basal bolus insulin therapy in the inpatient management of patients with type 2 diabetes undergoing general surgery (RABBIT 2 Surgery)

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Background and aims: This randomized multicenter trial compared the efficacy and safety of a basal/bolus regimen to sliding scale regular insulin (SSI)

in non-ICU patients undergoing general surgery. Study outcomes included differences in daily BG levels and a composite of hospital complications including postoperative wound infection, pneumonia, respiratory failure, acute renal failure, and bacteremia.
Materials and methods: A total of 211 patients (age: 58±11 yr, admission BG: 190±92 mg/dl, A1C: 7.72±2.2%, ±SD) with a BG between 140-400 mg/dl and a history of T2DM >3 months were randomized to glargine + glulisine (Gla+Glu, n=104) or SSI (n=107). Total daily dose of Gla+Glu was started at 0.5 U/kg, given half as glargine once daily and half as glulisine before meals. SSI was given 4 times/day for BG >140 mg/dl.
Results: The mean daily BG level after the 1st day of Gla+Glu vs. SSI was 145±32 mg/dl and 172±47 mg/dl, respectively, p<0.01. The percentages of BG readings <140 mg/dl were higher in Gla+Glu than SSI (53±30% vs 31±28%, p<0.001). We observed significant difference between groups in the frequency of the composite outcome (24.3% and 8.6% in the SSI and Gla+Glu, respectively; P=0.003). There were no differences in mortality (1% vs 1%); however, there were reductions with Gla+Glu as compared with SSI in wound infection (2.9% vs 10.3%), pneumonia (0% vs 2.8%), and acute renal failure (3.8% vs 10.3%), p=0.05, 0.24, 0.10. Compared to SSRI group, Gla+Glu reduced the number of post-surgical ICU admissions (19.6% vs 12.5%, p=0.159) and ICU length of stay (3.2±2 vs 1.2±0.6 days, p=0.003). A BG <70 mg/dl was reported in 23.1% of patients (1.9 % of BG readings) in the Gla+Glu and in 4.7% (0.3% of BG readings) in the SSI group, p<0.001; but only 3.8% of patients in the Gla+Glu and 0% in SSI had a BG <40 mg/dl (p=0.057).
Conclusion: In summary, treatment with glargine once daily plus glulisine before meals improved glycemic control and reduced hospital complications compared to SSI in general surgery patients with T2DM. Our study indicates that basal/bolus insulin regimen is preferable to SSI in the hospital management of general surgery patients with T2DM. NCT00596687.
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OP 41 Deregulation of fatty acid handling, obesity and diabetes

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Effect of different dietary fat quantity and quality on skeletal muscle fatty acid handling in subjects with the metabolic syndrome
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Background and aims: Insulin resistance is characterized by disturbances in lipid metabolism and increased fat storage in 'non-adipose tissues' like skeletal muscle (SM). The aim of this study was to investigate whether SM gene expression and the lipid content and the fatty acid (FA) profile of the SM lipids are affected by diets with different fat quantity and quality in subjects with the metabolic syndrome (MetS, NCEP criteria).
Materials and methods: 84 subjects (age 57.3±0.9y, BMI 30.9±0.4kg/m2, 42men/42women) were randomly assigned to one of four isoenergetic diets: high-SFA (HSFA); high-MUFA (HMUFA) and two low-fat, high-complex carbohydrate (LFHCC) diets, supplemented with 1.24 g/day of long chain n-3 PUFA (LCn-3) or control for 12 weeks. Insulin sensitivity (SI) was determined by a insulin modified intravenous glucose tolerance test. SM biopsies were taken before and after the intervention to determine expression of genes involved in lipid metabolism. In a subgroup (n=25, all men) muscle TAG, DAG, free FA (FFA), and phospholipid content, their fractional synthetic rate (FSR) as well as lipid composition were determined. The people in the subgroup consumed (before and after dietary intervention) a high-fat mixed meal (2.6MJ, 61E% fat) with 200 mg [U-13C]-palmitate added. Muscle biopsies were taken before and four hours after the meal. The FSR per lipid fraction was calculated by dividing the change in 13C-enrichment (at 4h postprandial minus baseline) in each fraction by the change in 13C-enrichment in the precursor pool (FFA). The study protocol was approved by the local Medical Ethical Committee of the Maastricht University.
Results: Expression of genes involved in lipogenesis (SREBP1c, SREBP2, ChREBP and ACC2) were downregulated after 12-weeks on HMUFA (mean fold change (FC) of -1.3) and on LFHCC LCn-3 (mean FC -1.7) in insulin resistant (IR) subjects (below the median of SI), whereas insulin sensitive (IS) subjects showed the opposite effect (mean FC +1.6 at both diets). HMUFA diet caused reduced DAG content (paired t-test p=0.027) and tended to decrease the FSR in TAG (p=0.055) and DAG (p=0.066). LFHCC LCn-3 diet reduced the muscle TAG content (p=0.032) and tended to increase percentage saturation of DAG (p=0.064).
Conclusion: Both HMUFA and LFHCC LCn-3 promoted a reduction of lipogenic genes in IR subjects with the MetS. In a subgroup HMUFA and LFHCC LCn-3 reduced DAG or TAG content, respectively, suggesting that these diets may reduce muscle fat accumulation by affecting the balance between fat storage and oxidation.
Supported by: DFN

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Adipokines promote lipotoxic effects of low levels of palmitic acid but not oleic acid by reducing fatty acid oxidation and increasing diacylglycerol
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Background and aims: Skeletal muscle insulin resistance is an early defect in the pathogenesis of type 2 diabetes mellitus. Numerous studies have shown that elevated plasma free fatty acid (FA) levels as well as intramyocellular lipid accumulation are positively correlated with the incidence of insulin resistance. Furthermore, it is accepted that adipose tissue functions as a secretory organ releasing various adipokines. Aim of this study was to investigate combined effects of adipokines with physiological concentrations of free FA on human skeletal muscle metabolism. Furthermore, possible differences of saturated and unsaturated FA were to be analysed.

Materials and methods: Differentiated primary human skeletal muscle cells (SkMC) were incubated with adipocyte-conditioned media (CM) for 24 h, while oleic acid (OA, 100 µmol/l) or palmitic acid (PA) were added for the final 18 h of incubation. Subsequently, SkMC were lysed for Western Blot analysis, incubated with ^{14}C-FA for FA oxidation, fixed for microscopic examination, or analysed using thin layer chromatography (TLC).
Results: Incubation of SkMC with CM increased the expression of FA transport protein CD36 (2-fold, n=3), while isolated adipokines failed to produce the same effect. Electron microscopic examination showed profound accumulation of lipid droplets after incubation with OA and CM, while there were no lipid droplets observed after treatment with PA alone or combined with CM. However, mitochondrial morphology was noticeably altered after treatment with PA. Analysis of the lipid droplet coating protein ADRP revealed a significantly increased expression after incubation with OA and CM (2-fold, n=6). FA oxidation was found to be reduced after incubation with PA and CM (by 72%), while incubation with CM (34%), OA (23%), and OA in combination with CM (33%, n≥5) caused a more moderate effect. Additionally, treatment of SkMC with a higher FA concentration (300 µmol/l) yielded a more severe reduction of FA oxidation by ~90% after treatment with PA and CM. TLC analysis revealed a significantly increased diacylglycerol (DAG) content (3-fold, n≥3) after incubation with PA and CM.
Conclusion: The results of this study indicate that physiological levels of FA, which are described to not affect SkMC metabolism, have deleterious effects in combination with adipokines. Hence, it may be speculated that adipokines rather than FA may play a more crucial role in mediating insulin resistance, since they not only increase FA uptake but also seem to interfere with FA metabolism. Furthermore, these results support the notion that saturated FA like PA are more detrimental than unsaturated FA like OA. While OA seems to increase the potential of the cell to store excess lipids in lipid droplets by increasing the expression of ADRP, PA seems to impair mitochondrial integrity and in combination with adipokines leads to incomplete FA oxidation and concurrent accumulation of DAG. Thus it may be assumed that already at an early stage of weight gain, when lipolysis has not yet contributed to increased plasma free FA levels, there might be lipotoxic damage to skeletal muscle cells.

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Splanchnic balance of free fatty acids, endocannabinoids and lipids in subjects with NAFLD

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Background and aims: Animal studies suggest that endocannabinoids could contribute to the development of non-alcoholic fatty liver disease (NAFLD). In addition, NAFLD has shown to be associated with multiple changes in lipid concentrations in liver biopsies. There are no data on splanchnic free fatty acid, glycerol, ketone body, endocannabinoid and lipid fluxes *in vivo* in subjects with NAFLD.
Materials and methods: We performed hepatic venous catheterization studies in combination with [$^2H_2$]palmitate infusion in the fasting state and during a low-dose insulin infusion (0.5 mU/kg min) in nine subjects with various degrees of hepatic steatosis as determined using liver biopsy. Splanchnic balance of endocannabinoids and individual lipids was determined using Ultra Performance Liquid Chromatography coupled to mass spectrometry.
Results: Splanchnic free fatty acid (FFA) extraction during the euglycemic hyperinsulinemia correlated with liver fat content (r=0.75, p=0.05). Concentrations of the endocannabinoid anandamide were higher in arterialized (91±33 µmol/l basally) than in hepatic venous (51±19 µmol/l, p<0.05) plasma. Fasting arterial (r=0.72, p=0.031) and hepatic venous (r=0.70, p=0.037) concentrations of anandamide were positively related to liver fat content. Analysis of fluxes of 85 different triglycerides showed that the fatty liver overproduces saturated triglycerides. In the plasma FFA fraction in the basal state, the relative amounts of palmitoleate and linoleate were lower and those of stearate and oleate higher in the hepatic vein than in the artery. Absolute concentrations of all non-triglyceride lipids were comparable in arterialized venous plasma and the hepatic vein both in the basal and insulin-stimulated states.
Conclusion: FFA extraction during hyperinsulinemia correlates with liver fat content, consistent with data showing defects in insulin action on lipolysis to contribute to liver fat. The human fatty liver takes up anandamide and overproduces triacylglycerols containing saturated fatty acids, which might reflect increased *de novo* lipogenesis.
Supported by: Academy of Finland, Sigrid Juselius Foundation, HEPADIP, FDRA, OF, RAF, HBRF

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Fatty acid class influences spillover from chylomicrons into plasma nonesterified fatty acids

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Background and aims: The mechanism by which diets high in polyunsaturated fatty acids (PUFA) reduce cardiovascular risk is not known. Lipoprotein lipase (LPL) mediates dietary fat storage in adipose tissue via its action on chylomicron triglycerides, but a portion of LPL-generated fatty acids are released directly into the plasma nonesterified fatty acid (NEFA) pool via a process known as spillover. The present study was undertaken to determine whether there are differences in spillover among different classes of fatty acids.
Materials and methods: Twelve lean, healthy adults were studied after a 5 day controlled diet and an overnight fast. Volunteers consumed ~15 mL of a liquid meal, consisting of a commercial dietary supplement and [1-^{13}C] tripalmitin, [1-^{13}C] triolein and [9,10-^{3}H] trilinolein, every 15 minutes for 6 hours. [1-^{14}C] palmitate, [U-^{13}C] oleate and [U-^{13}C] linoleate were infused intravenously for 2 h. Blood samples were taken for NEFA concentrations, tracer enrichment and specific activity, as well as chylomicron and total triglyceride concentration. Systemic rate of appearance (R_a) and spillover were calculated for each fatty acid using steady state assumptions.
Results: Total NEFA concentrations were 114±11 µmol/L during meal absorption. Plasma oleate concentration was significantly higher than palmitate or linoleate concentrations (41±5 vs 23±3 and 29±3 µmol/L, respectively; p<0.05 for both). The R_a of palmitate was significantly lower than the R_a of either linoleate or oleate (0.4±0.03 vs 0.8±0.07 and 0.8±0.06 µmol/kg/min, respectively, both p < 0.001). Clearance of linoleate was higher than that of either palmitate or oleate (32±3 vs 21±3 and 22±2 mL/kg/min, respectively; p < 0.03 for both). Fractional spillover among the 3 groups was 40±4% v 38±3% v 23±2% for palmitate, oleate and linoleate respectively. The difference between linoleate compared to palmitate and oleate was significant (p<0.01 for both comparisons). There was no difference between palmitate and oleate spillover.
Conclusion: These data show a significant difference in spillover of chylomicron linoleate during meal absorption in normal subjects compared to the other two most abundant dietary fatty acids. The low spillover of linoleate indicates comparatively efficient storage of this fatty acid and thus less availability for ectopic fat accumulation in tissues such as liver and skeletal muscle. The relationship between this finding and the apparent reduction in cardiovascular risk associated with PUFA-enriched diets will require further study.
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OP 42 Inflammation and metabolism

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Resistin and adipocyte fatty acid binding protein are linked in type 2 diabetes mellitus and atherosclerosis

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Background and aims: Systemic atherosclerosis is the co-morbidity of type 2 diabetes mellitus (T2D). Patients with T2D have increased body weight and adipokine production. The adipokines resistin and adipocyte fatty acid binding protein (AFABP) have each been linked to T2D and atherosclerosis. Recently, a direct influence of resistin on AFABP has been demonstrated in cell culture of human endothelial cells, whereas respective experiments in knockout mice failed. We investigated the interrelationship of resistin and AFAB in a cohort study of human individuals.
Materials and methods: Resistin and AFABP serum levels were investigated in 168 patients (61 female, 107 male) with systemic atherosclerosis. All patients suffered from peripheral arterial disease (PAD); 76 patients showed additional coronary or cerebral artery disease. Oral glucose tolerance tests (oGTT) were performed in all patients. Resistin and AFABP were obtained by commercially available ELISA (BioVendor, Modrice, Czech Republic). Inter-assay coefficient of variation (CV) and intra-assay CV were 7.8% and 4.8% for resistin and 6.5%, and 2.9% for AFABP. Students' unpaired t-test, univariate and multivariate regression modeling were applied as appropriate. Skewed data were lg10 transformed to render the distribution normal for parametric tests. Normal data are given as mean±STD, non-parametric data as median (25%;75%). In multivariate analysis, change of beta (D-beta) over 10%, were considered as confounding.
Results: According to oGTT, we had 51 subjects with normal glucose metabolism (NGM), 35 subjects with pre-diabetes (PRED, impaired fasting glucose/glucose tolerance), and 82 subjects with overt T2D. Resistin levels did not differ (NGM vs PRED vs T2D 6.5±1.7 vs 7.02±2.9 vs 7.26±2.5 ng/ml; p=0.195). AFABP levels were significantly higher in diabetes (NGM vs PRED vs T2D 29 (16;39) vs 30 (20;41) vs 33 (23;45) ng/ml; p=0.035). By univariate regression resistin was associated with AFABP in all patients (beta=0.308; p<0.001). Subgroup analysis revealed that the association was based on T2D (beta=0.392; p<0.001). Since recent papers have suggested an association between diabetes, hypertension and resistin in mouse models, data of the T2D subgroup were tested for confounding by multivariate modeling. Linear regression revealed that the effect of resistin on AFABP is not affected by the subjects' blood pressure (systolic D-beta=1%; diastolic D-beta=2%), but is attenuated with increasing number of anti-hypertensive medication taken by the patient (D-beta=11%). Vice versa, alternative linear regression models showed that the association of AFABP on resistin is not affected by the subjects' blood pressure (systolic D-beta=1%; diastolic D-beta=0%) or hypertension medication (D-beta=1%). Furthermore, this association of resistin on AFABP or AFABP on resistin was not attenuated by body mass index in multivariate models (D-beta=1% and D-beta=4%). The severity of atherosclerosis showed no effect on resistin and AFABP levels.
Conclusion: This is the first study to demonstrate an association of the adipokines resistin and AFABP in patients with systemic atherosclerosis. This association was due to the existence of diabetes, but not pre-diabetes. Whether hypertension is interrelated with resistin and AFAB in patients with or without diabetes needs to be investigated.

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Asymmetric dimethylarginine does not contribute to endothelial dysfunction in subjects with abnormalities of glucose regulation

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Background and aims: Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of endothelial nitric oxide synthase (eNOS), has been associated with endothelial dysfunction and atherosclerosis. Increased plasma levels of ADMA have been described in diabetic subjects with nephropathy or cardiovascular disease. Studies assessing ADMA levels in people with uncomplicated type 1 or type 2 diabetes report conflicting results.
Materials and methods: Circulating levels of ADMA, SDMA (symmetrical dimethylarginine) and L-arginine (L-arg) together with brachial artery endothelium-dependent flow-mediated dilation (FMD) and endothelium-independent dilation by 25 μg sublingual glyceryl trinitrate (GTN) were evaluated in 26 subjects with normal glucose tolerance (NGT), 34 with pre-diabetes (IFG or IGT, pre-DM) and 18 newly diagnosed type 2 diabetics (newT2DM) identified through OGTT. Plasma concentrations of ADMA, SDMA and L-arg were determined simultaneously by high-performance liquid chromatography. FMD and GTN were assessed by high-resolution ultrasound and computerized edge detection system.
Results: Groups showed similar distribution for gender, smoking habits, BMI, waist circumference, dBP, total and LDL cholesterol, apoA1 and apoB, fibrinogen, and fasting insulin. Namely, there were no differences in eGFR and cystatin C. Age (55±5, 53±8 vs 48±9 years, p=0.01), fasting and post-load OGTT glucose, glucose area under the OGTT curve (AUCgluc), HbA1c (6.0±0.4, 6.5±0.6 vs 5.5±0.4%, p<0.0001) and triglycerides were higher in pre-DM and newT2DM than in NGT; HDL cholesterol was lower. In pre-DM, sBP (127±13) was in between NGT (118±15) and newT2DM (137±15 mmHg, p=0.0005). GTN decreased (Δ% 9.9±3.4, 8.8±3.3 and 7.4±3.9, m±sd; p=0.081) with significant differences between NGT and newT2DM (p=0.025). FMD was lower in newT2DM (Δ% 4.4±3.3, m±sd) and in pre-DM (Δ% 6.0±2.8) compared with NGT (Δ% 7.9±3.6, p=0.0017). L-arg levels were similar in NGT (97.5±20.0) and pre-DM (97.1±20.6), lower in newT2DM (81.2±18.9 μmol/l, p=0.015). ADMA progressively reduced from NGT (1.33±0.96 μmol/l) to pre-DM (1.02±0.79 μmol/l, p=0.14 vs NGT) and newT2DM (0.72±0.53 μmol/l, p=0.017 vs NGT; ANOVA, p=0.05). SDMA was similar in NGT (1.78±0.74) and pre-DM (1.56±1.02, p=0.31), reduced in newT2DM (0.97±0.35 μmol/l, p=0.002 vs NGT, p=0.02 vs pre-DM; ANOVA, p=0.006). No association was observed between ADMA (or SDMA) and eGFR or cystatin C. No correlation emerged between ADMA and FMD (r=0.14, p=0.23) with a weak one between ADMA e GTN (r=0.29, p=0.014). By multiple regression, AUCgluc (p=0.002) and sBP (p=0.047), but not ADMA were inversely related with FMD. AUCgluc, inversely, (p=0.024) and ADMA (0.044) correlated with GTN.
Conclusion: We suggest that uncomplicated newT2DM and subjects with pre-DM have lower circulating ADMA than nondiabetic control subjects, in presence of impaired endothelium-dependent flow-mediated dilation. ADMA levels are not related to endothelial function. In these subjects with early abnormalities of glucose regulation, endothelial dysfunction seems not a result of eNOS inhibition by ADMA.

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Vascular inflammation stratified by C-reactive protein and LDL-cholesterol levels: analysis with ^{18}F-Fluorodeoxyglucose positron emission tomography

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Background and aims: ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET) is a promising imaging technique for the assessment of vascular inflammation within atherosclerotic plaques. Inflammatory biomarkers, such as high sensitivity C-reactive protein (hsCRP), have been suggested as independent predictors of cardiovascular events that add prognostic information beyond conventional risk factors. Recently, the justification for the use of statins in primary prevention: an intervention trial evaluating rosuvastatin (*JUPITER*) study has demonstrated that rosuvastatin significantly reduces the incidence of major cardiovascular events in asymptomatic individuals with low LDL-C levels, but increased hsCRP levels, a population that is currently not recommended to receive statin therapy.
Materials and methods: We examined vascular inflammation, represented as the target-to-background ratio (TBR) measured using FDG-PET scans in 120 healthy subjects without history of cardiovascular diseases, who had been stratified into four groups according to hsCRP *(cut-point, 2mg/L)* and low-density lipoprotein cholesterol (LDL-C) levels *(cut-point, 130mg/dL)*. We also determined the correlation between circulating levels of other emerging inflammatory markers, such as lipoprotein-associated phospholipase A_2 (Lp-PLA_2), monocyte chemoattractant protein-1 (MCP-1), and vascular inflammation assessed by FDG-PET. Lastly, we compared the factors which determine TBR and carotid intima-media thickness (IMT).

Results: Maximum TBR levels of the high hsCRP, low LDL-C group were significantly higher than those of the low hsCRP, low LDL-C or low hsCRP, high LDL-C group, even though there were no significant differences in IMT. TBR values were associated with various cardiovascular risk factors, including hsCRP, which had the strongest positive correlation with TBR. However, Lp-PLA_2 or MCP-1 levels were not independently associated with TBR values. Multiple stepwise regression analyses showed that hsCRP and diastolic blood pressure were independent decisive factors for maximum TBR, whereas age, diastolic blood pressure, and LDL-C were factors which determined the maximum IMT.
Conclusion: Vascular inflammation measured using FDG-PET was increased in healthy individuals without hyperlipidemia, but with elevated hsCRP.

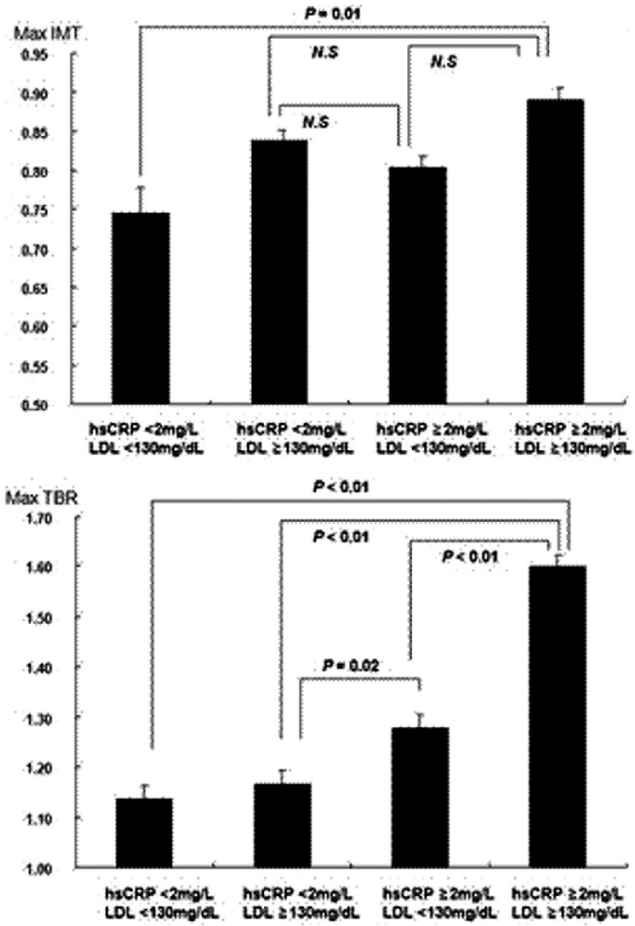

Figure 1. Maximum intima-media thickness (IMT) (A) and maximum target-to-background ratios (TBR) (B) by stratified groups according to high sensitivity C-reactive protein (hsCRP) and low-density lipoprotein cholesterol (LDL-C) levels. P-value represents pair-wise comparison based on Bonferroni's multiple comparison procedure under analysis of covariance (ANCOVA) adjusted for age, gender and BMI. *N.S*, non-significant.

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High serum LPS-activity is associated with features of the metabolic syndrome in patients with type 1 diabetes

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Background and aims: Long duration of diabetes, poor glycemic control and the metabolic syndrome (MetS) increase the risk for diabetic complications (retinopathy, neuropathy and nephropathy). We have recently shown that elevated serum levels of bacterial endotoxins are associated with the development of diabetic kidney disease in Finnish Type 1 diabetic (T1D) patients. In addition to kidney failure, lipopolysaccharide (LPS) infusion in human or in mice induces also insulin resistance, fasting hyperglycemia, and obesity, which all are features of the MetS. In the present study, we wanted to investigate whether serum LPS-activity is associated with MetS in T1D patients with normal albumin excretion.
Materials and methods: Serum LPS activity was analysed in 624 T1D patients and 220 nondiabetic control subjects (Limulus amoebocyte lysate chromogenic end point assay, Hycult Biotechnology). T1D patients were divided into quartiles according to their LPS activity. MetS was assessed according to National Cholesterol Education Program (NCEP) criteria which included waist circumference, triglycerides, HDL-cholesterol, and blood pressure or antihypertensive medication. All patients fulfilled the criteria for hyperglycemia. Three out of five criteria were required for the diagnosis of MetS. A metabolic score (1-5) was calculated based on the number of criteria each patient fulfilled. Data is presented as mean (standard deviation) or median [inter quartile range] as appropriate.
Results: LPS was significantly higher in patients with T1D than healthy controls [57 (50-69) vs. 53 (39-68), p≤0.001]. In T1D patients, comparison was made between the highest (q4) and lowest (q1) LPS quartiles. Patients in q4 had a higher HbA_{1c}, BMI, waist, triglycerides, cholesterol, diastolic blood pressure and lower HDL-cholesterol and insulin sensitivity (eGDR) compared to patients in q1. The overall frequency of MetS was 28% among all T1D patients. Patients belonging to the highest LPS quartile q4 had significantly higher frequency of MetS compared to patients in q1 (Table 1).
Conclusion: In the present study, we show that about one third of T1D patients with normal albumin excretion fullfill the criteria for MetS. Features of the MetS are more often found in T1D patients with high serum LPS-activity. These results indicate that Gram-negative bacterial infections could also play a significant role in the development of MetS. We believe that MetS patients with elevated levels of bacterial endotoxins may carry the highest risk for the development of not only micro- but also macrovascular complications.

Table 1. Highest and lowest LPS quartile

	q1 (<50.1)	q4 (>69.4)
N (M/F)	146 (57/89)	147 (76/71)†
Age	47 (39-55)	39 (31-50)*
Age at Onset	18 (11-25)	13 (9-21)*
HbA_{1C} (%)	7.4±1.4	7.9±1.2*
Systolic BP (mmHg)	137±18	136±16
Diastolic BP (mmHg)	76±9	81±9*
Antihypertensive medication (%)	36	27
Waist (M) (cm)	91±9	96±12†
Waist (F) (cm)	80±9	88±13*
Triglycerides (mmol/l)	0.7 (0.6-0.8)	1.4 (1.2-1.9)*
HDL-cholesterol (M) (mmol/l)	1.6±0.5	1.3±0.4*
HDL-cholesterol (F) (mmol/l)	1.8±0.4	1.6±0.4†
Metabolic syndrome (%)	16	46*

All values are compaired to q1. *p≤0.001; †p≤0.05

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OP 43 New oral agents

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Dapagliflozin vs glipizide in patients with type 2 diabetes mellitus inadequately controlled on metformin: 52-week results of a double-blind, randomised, controlled trial

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Background and aims: Glipizide (GLIP) is commonly used as an add-on to metformin (MET), but is associated with weight gain and hypoglycaemia. Dapagliflozin (DAPA) is a selective inhibitor of sodium-glucose cotransporter 2 that inhibits renal glucose reabsorption in an insulin-independent manner. DAPA is a potential therapy to reduce hyperglycaemia in T2DM, and has been associated with weight loss. We tested the efficacy, safety and tolerability of DAPA vs GLIP as add-on to MET in patients with T2DM.
Materials and methods: This randomized, double-blind, active-controlled, parallel-group, multicentre trial (D1690C00004) included patients with T2DM inadequately controlled with oral antidiabetic drugs (OADs) including MET (HbA_{1c} 6.5-10.0%). Prior to randomization, as needed, OADs other than MET were discontinued and MET dose was up-titrated to the nearest of 1500, 2000, or 2500 mg/day, before an 8-week (wk) stabilization period. After a 2-wk placebo lead-in, patients (≥18 years) were randomized to DAPA (n=406, starting 2.5 mg/d) or GLIP (n=408, starting 5 mg/d) added to open-label MET for 52 wk. For the first 18-wk study drugs were up-titrated (GLIP to ≤20 mg/d; DAPA to ≤10 mg/d) until fasting plasma glucose <6.1 mmol/L or to the max tolerated dose. The dose at the end of titration was maintained for a further 34 wk. Down-titration was allowed at any point in cases of recurrent hypoglycaemia. Primary endpoint was change from baseline in HbA_{1c} at 52 wk, tested for non-inferiority of DAPA vs GLIP with a predefined margin of 0.35%. Secondary endpoints included change in body weight and number of subjects reporting hypoglycaemic episodes. There was no preplanned statistical analysis for other adverse events (AEs).
Results: Mean baseline HbA_{1c} was 7.72%. At the end of the titration period, 86.9% of DAPA and 72.5% of GLIP patients were taking max doses. Adjusted mean changes from baseline in HbA_{1c} at 52 wk were -0.52% (95% CI -0.60, -0.44) for DAPA and -0.52% (95% CI -0.60, -0.44) for GLIP (difference [95% CI] = 0.00 [-0.11, 0.11]), confirming non-inferiority. DAPA led to weight loss (change from baseline at 52 wk -3.2 kg) vs weight gain (1.4 kg) with GLIP (difference [95% CI] = -4.7 kg [-5.1, -4.2]; p<0.0001). Significantly more patients experienced weight loss of ≥5% from baseline with DAPA (33.3%) vs GLIP (2.5%) (p<0.0001). There were reductions in systolic and diastolic blood pressure (nominal p<0.0001) and improvement in HDL (nominal p<0.0001) in DAPA vs GLIP. More patients had hypoglycaemic episodes with GLIP (40.8%) vs DAPA (3.5%) (p<0.0001). Overall AEs were similar across groups. Serious AEs were 8.6% in DAPA and 11.3% in GLIP. Actively solicited events suggestive of urinary tract infection (UTI) were 10.8% and 6.4% for DAPA vs GLIP. Actively solicited events suggestive of genital infection (GI) were 12.3% (5.3% male, 21.1% female) and 2.7% (0.4% male, 5.4% female) for DAPA vs GLIP. One UTI led to discontinuation in each of the DAPA and GLIP arms. Three cases of GI led to discontinuation in the DAPA arm.
Conclusion: In T2DM inadequately controlled with OADs including MET, the addition of DAPA was non-inferior to GLIP in improving HbA_{1c} at 52 wk, resulted in significant weight loss and significantly fewer hypoglycaemic episodes vs GLIP, and was generally well tolerated, with a tendency to more UTIs and GIs.
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MK-0941, a novel glucokinase activator (GKA), lowers HbA_{1c} in type 2 diabetes (T2DM) but lacks glycaemic durability

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Background and aims: GKAs are allosteric activators of the GK enzyme that bind to the same region as naturally-occurring GK activating mutations in humans. GKAs are being developed as novel potential treatments for patients (pts) with T2DM. Preclinical studies with the GKA MK-0941 showed robust glucose-lowering effects both acutely and chronically (up to 9 mo) in association with hypoglycaemia (Hypo) in fasted and fed non-diabetic animals. MK-0941 was evaluated in Phase (Ph) I studies, including those up to 4 wks in pts with T2DM, as monotherapy, add-on to metformin (MET), and add-on to insulin glargine (IG). In these studies, MK-0941 was generally well-tolerated, with no significant treatment-related effects on ECGs, vital signs, or lab measures, and was shown to have a duration of action of ~4 hrs. On the background of IG, MK-0941 showed robust glucose-lowering, with a reduction in 24-hr weighted mean glucose (WMG) of ~2.7 mmol/L relative to placebo (pbo). These data supported continuing development of MK-0941 with Ph II studies in pts with T2DM.
Materials and methods: MK-0941 was evaluated in 3 randomized, double-blind Ph II trials:
• 007, a 54-wk, pbo-controlled study in 587 pts on ongoing IG therapy (± MET ≥1500 mg/d), comprising an initial 14-wk, dose-ranging (10 - 40 mg TID) period followed by an additional 40-wk period during which all pts were to be up-titrated as tolerated to 40 mg or pbo TID;
• 017, a 6-wk, active-controlled study in 143 pts on going MET (≥1500 mg/d), with patients randomized to MK-0941 (up to 40 mg TID) or glimepiride (GLIM; up to 8 mg QD);
• 018, a 20-wk, pbo-controlled, MK-0941 dose-titration (up to 40 mg TID) study in 68 pts on ongoing IG therapy.
The primary endpoint was change from baseline in HbA_{1c} (007 and 018) or 24-hr WMG (017).
Results: In 007, at Wk 14, all MK-0941 doses studied significantly improved HbA_{1c} and 2-hr postmeal glucose (PMG) vs. pbo, with maximal pbo-subtracted changes from baseline in HbA_{1c} (baseline HbA_{1c} ~9.0%) and 2-hr PMG of -0.8% and -2.1 mmol/L, respectively. No significant effect on FPG was observed at any dose vs. pbo. Unexpectedly, efficacy results up to 30 wks demonstrated a lack of durability in glycaemic control (despite dose up-titration of MK-0941 after Wk 14), a phenomenon not predicted based on earlier studies. MK-0941 was associated with an increased incidence of Hypo relative to pbo that was, in part, managed with down-titration of MK-0941. In 017, 6-wk treatment with MK-0941 or GLIM added to ongoing MET resulted in similar changes in 24-hr WMG from baseline and incidences of Hypo. In 007 and 017, statistically significant increases in serum triglycerides (~15% median percent increase from baseline) and the proportion of pts meeting criteria for predefined limits of change for BP measures were observed. These safety findings were not seen in preclinical or Ph I studies. In 018, change in HbA_{1c} from baseline at Wk 20 was not significantly different between MK-0941 and pbo when added to IG. The incidence of Hypo was numerically higher with MK-0941.
Conclusion: In Ph I studies, MK-0941 showed promise as an investigational agent for T2DM. This expectation was not borne out in 3 longer-term, Ph II studies. It is unknown if the efficacy and safety profiles observed with MK-0941 were compound-specific or mechanism-based. In light of the above, a better understanding of the GK mechanism and its downstream metabolic effects are needed to determine whether GK activation is a viable treatment target.
Supported by: Merck

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A selective GPR40 agonist, TAK-875, augments glucose-dependent insulin secretion without affecting glucagon secretion in isolated rat and human islets

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Background and aims: GPR40 is a G protein-coupled receptor dominantly expressed in pancreatic β cells, and is involved in free fatty acid-induced insulin secretion. TAK-875 is a GPR40-selective agonist that improves glucose control in type-2 diabetic animal models by stimulation of glucose-dependent insulin secretion. We examined the effects of TAK-875 on insulin and glucagon secretion, and on intracellular Ca^{2+} ($[Ca^{2+}]_i$) in pancreatic β- and α-cells using both human and rat intact islets.
Materials and methods: Rat and human islets were isolated by collagenase digestion. Secreted insulin and glucagon were measured using radioimmunoassay. Gene expression levels were quantified by TaqMan PCR. For $[Ca^{2+}]_i$ measurement, isolated islets were loaded with fluorescent indicator fluo-4-AM and monitored by confocal microscopy during perifusion experiments.
Results: In static incubation, TAK-875 augmented insulin secretion from rat islets at high (16 mmol/l) but not at low (1 mmol/l) glucose. The glucose-de-

pendent insulinotropic action of TAK-875 was also shown in islet perifusion experiment: TAK-875 enhanced both 1st and 2nd phase insulin secretion at high glucose but was without effect at low glucose. In human islets, expression of GPR40 was comparable to that of GLP-1R or ABCC8 (SUR1), and TAK-875 enhanced glucose-dependent insulin secretion to the same extent as GLP-1 in static incubation experiment. In both rat and human islets TAK-875 was without effect on glucagon secretion at both low and high glucose. Measurements of $[Ca^{2+}]_i$ in intact rat and human islets showed that TAK-875 enhanced glucose-induced $[Ca^{2+}]_i$ in β cells. In contrast to β cells, α cells showed oscillatory $[Ca^{2+}]_i$ response at low glucose which was suppressed by high glucose concentration. The addition of TAK-875 at high glucose did not affect α-cell oscillatory $[Ca^{2+}]_i$ in rat islets, whereas it augmented the inhibitory effect of glucose in human islets.
Conclusion: These data indicate that TAK-875 potentiates glucose-dependent insulin secretion via direct stimulation of $[Ca^{2+}]_i$ in β cells of both rat and human islets. TAK-875 does not increase glucagon secretion or $[Ca^{2+}]_i$ in both rat and human α-cells. We conclude that the glucose-lowering action of TAK-875 principally results from its capacity to stimulate insulin secretion. The fact that it only stimulates insulin secretion at elevated glucose levels without affecting glucagon secretion may offer additional advantages by minimizing the risk of hypoglycaemia.

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ZGN-201 (ZGN), a methionine aminopeptidase 2 (MetAP2) inhibitor, durably eliminates excess body fat in obese mice through regulation of fat metabolism and food intake
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Background and aims: MetAP2 inhibitor treatment reduces body weight (BW), reduces food intake, and increases fat oxidation in obese mice; however the mechanism(s) leading to weight loss have not been elaborated. We evaluated the effects of a 9 month treatment with ZGN on body weight and metabolic parameters in obese mice.
Materials and methods: Age-matched male C57BL/6J mice were maintained on standard chow or a 60% high fat diet for 12 weeks to induce obesity (DIO; mean BW 39.9 g) prior to treatment for 9 months. After matching on the basis of body weight, animals were assigned to either HFD (DIO; n=15 mice) or HFD supplemented with ZGN-201 to provide a daily dose of ~1 mg/kg (ZGN; n=15 mice). A group of lean age-matched chow-fed mice were studied for comparison (CHOW; n=15 mice). Food consumption was assessed every two days for groups of three animals per cage. Body weights were assessed for each animal every other day. Plasma glucose and beta-hydroxybutyrate concentrations were measured by standard colorimetric assays. Insulin was measured by ELISA. Gene expression analysis was performed in liver using quantitative RQ-PCR, and levels were corrected for expression of 18S rRNA.
Results: During the first 4 weeks of treatment with 1 mg/kg ZGN p.o., mice lost all excess BW (loss of 9.4±0.7g vs a gain of 5.0±0.4g for control (DIO) mice, p<0.01), driven by a 30% reduction in food intake during days 3 - 12 of treatment (p< 0.01). Once a BW nadir was reached on day 28 (23% BW loss, p<0.01), food intake returned to a level 13 percent below DIO (0.81g vs. 0.94g/mouse-day, p<0.01) and was stable for the following 8 months, during which time the ZGN treated animals remained weight stable. Following 9 months, BW of ZGN mice were 43% lower than DIO (32.5±0.8 vs 57.1±1.7g, p<0.01). Fasting plasma glucose (6.8±0.4 vs 11.2±0.8 mmol/L, p<0.01) and insulin (34±17 vs 380±34 pmol/L, p<0.01) were reduced by ZGN and plasma β-hydroxybutyrate was increased (1.5±0.1 vs 1.0±0.1 mEq/L, p<0.05) relative to DIO, a consistent feature of MetAP2 treatment. Gene expression analysis revealed a down-regulation of key lipid synthesis genes in liver for ZGN vs DIO or age-matched CHOW mice. The insulin- and carbohydrate-responsive genes acetyl CoA carboxylase 1 and 2, fatty acid synthase, stearoyl CoA desaturase 1, and SREBP1c all were down-regulated by 58, 77, 80, 99, and 74%, respectively vs. DIO (all p<0.005), and by 31, 71, 74, 98, and 43%, respectively vs. CHOW (ZGN vs. CHOW, all p<0.05).
Conclusion: Hyperinsulinemia in the setting of diet-induced obesity activates fatty acid biosynthesis and transport pathways, reduces adipose lipolysis, and suppresses ketone body synthesis leading to enhanced triglyceride storage. MetAP2 inhibition appears to be well-tolerated and shows promise as a strategy to reverse hyperinsulinemia and other obesity-associated metabolic adaptations while driving rapid weight loss.

OP 44 Impact of education on glycaemic outcome

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Patient education: impact on care outcomes, resources consumption and absenteeism. International Diabetes Management Practices Study (IDMPS) data
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Background and aims: Achievement of diabetes treatment goals greatly depends on the patient´s active and efficient participation in the control and treatment of the disease. Even when evidence has demonstrated the value of education to obtain such participation, in many places education is considered an additional cost rather than an important component of diabetes care. This study measures diabetes care indicators and resources consumption in a population of educated and non educated persons with type 2 diabetes.
Materials and methods: The IDMPS is an international, multicenter, observational study performed in 27 countries within Africa, Asia, Eastern Europe, Middle East and Latin America. Data were collected from people with type 1 and 2 diabetes (≥18 years) seen in current medical practice in yearly cycles (2-week cross-sectional recruitment period followed by a 9-month longitudinal period for type 2 patients) for 5 years. IDMPS was performed in compliance with the Helsinki Declaration and Good Clinical Practice Standards. We currently report and compare results from 11384 people with type 2 diabetes (educated *vs.* non educated, 5692 in each group, paired by age, gender and diabetes duration) recruited during the second cross-sectional period (November and December 2006). Data were analyzed using the Wilcoxon and the chi^2 tests for continuous and categorical variables, respectively.
Results: The mean age ± SD for both groups was 57.7 ± 11.1 (53.1% female), with an average BMI of 28 ± 5.2 and 8 ± 7 years of diabetes duration. Educated patients had significantly (p< 0.001) higher figures of people with normal BMI (28.3 *vs.* 24.4%), diastolic BP <80 mm Hg (36.5 *vs.* 32.3%), HbA1c < 7.0% (38.1 *vs.* 35.8%), LDL-c < 100 mg/dL (39.5 *vs.* 33%) and triglyceride < 150 mg/dL (52.7 *vs.* 49%). The percentage of complications was low in both groups (<20%), but the educated group had significantly lower values of people with proteinuria (16.6 *vs.* 18%) and foot ulcer (2.4 *vs.* 3.5%). Visits of educated patients to specialists increased by 21%, as well as insulin treatment (40%), fasting (10%) and postprandial (52%) SMBG performance. Absenteeism was 15% lower in educated patients.
Conclusion: These data, one of the largest reported on education of people with type 2 diabetes, demonstrate that education can significantly improve treatment outcomes, with a low increase in resources consumption and a decrease in absenteeism. They also show that education is an efficient tool to improve diabetes care quality with low impact on resources consumption and additional positive impact on productivity, particularly important for developing countries that bear the heaviest part of the global diabetes epidemic.
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The effects of a structured educational programme (DAFNE) for individuals with type 1 diabetes on DKA admissions
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Background and aims: In the UK national diabetes audit 2007/2008, around 10% of people with type 1 diabetes had an episode of Diabetic Ketoacidosis (DKA) within 5 years. Structured education programmes for individuals with Type 1 diabetes are acknowledged as an effective means of delivering skills based training to improve knowledge of diabetes. They aim to influence behaviour and to optimise self management and reduce negative outcomes. Locally we deliver DAFNE, Dose Adjustment for Normal Eating, as a 5 day programme for individuals with type 1 diabetes. DAFNE was introduced in our centre in 2005. The aim of our study was to assess the effects of attend-

ing DAFNE on DKA admissions to an acute University Teaching Hospital in the UK.
Materials and methods: A retrospective review of all individuals with type 1 diabetes who attended a DAFNE course between Oct 2005 and July 2008. Only patients who lived locally and subsequently would have been admitted acutely to our hospital in a case of DKA were selected. We used the hospital coding system to detect all DKA admissions for the same group between Oct 2002 and July 2009.
Results: A total of 236 patients attended the DAFNE course. DKA admissions are summarized in table 1. DKA admission rate in the first year before DAFNE was 0.113 admissions/person/year (95% CI=0.073, 0.165) compared to 0.069 admissions/person/year (95% CI= 0.039, 0.112) in the first year after DAFNE. Relative risk reduction 39% (95% CI= 0, 78%); P= 0.05. For the group of patients with any DKA admission (n= 24), median duration of follow up of 24 months (range 15 - 44) before and after attending DAFNE, DKA admission rate was 2.375 admissions/person/year (95% CI= 1.789, 3.0777) before Vs 1.166 admissions/person/year (95% CI= 0.775, 1.686) after DAFNE. 212 patients had no DKA admissions before or after DAFNE within the same period of follow up.
Conclusion: Attending structured education (DAFNE) resulted in significant reduction in hospital DKA admission rate for individuals with type 1 diabetes. This suggests that offering a structured education might be a productive strategy in reducing DKA admissions in type 1 diabetes.

Table 1. DKA admissions in relation to DAFNE

Time in relation to DAFNE course	2nd year before	1st year before	1st yr after	2nd yr after
No. of DKA admissions	31	26	16	11
No. of patients with data available	217	230	236	163
DKA admissions per group per year	14.2%	11.3%	6.7%	6.7%

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PRODIACOR: Educative interventions improve clinical and metabolic outcomes and optimise treatment costs in an Argentinean population with type 2 diabetes

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Background and aims: PRODIACOR is a randomized controlled clinical trial implemented in a primary care setting (Corrientes city, Argentina) aimed at measuring the impact of educative interventions upon quality of care of people with type 2 diabetes (T2DM) and to measure the cost-effectiveness of such interventions.
Materials and methods: 36 primary care physicians and 468 persons with T2DM were randomized at physician level and allocated to 4 groups: 1) patients but not physicians received an education programme, 2) physicians but not patients received an education programme, 3) both physicians and patients received an education programme, and 4) control group (physicians/patients received no education but education material and data feedback). Patients from all groups received complete coverage of drugs and supplies; clinical, metabolic and therapeutic indicators were recorded. Educated physicians attended 4 interactive theoretical-practical modules and received a manual with all the algorithms for diagnosis, control and treatment of T2DM. Educated patient attended 4 weekly teaching units and a reinforcing session after 6 weeks, with a focus at improving health behaviour. Educational material included an individual log-booklet to record the self-monitored data (blood glucose and body weight) and a book with the main contents of the programme. Every patient - irrespective of his group allocation - received a check-book which served 2 purposes: a) as a reminder system for medical visits and laboratory test performance, and b) as a data collection system (record of laboratory tests, consultations or prescriptions for drugs or devices). Physicians monitored and recorded clinical data and data collection was monitored twice a year. We currently report baseline and 3-year follow-up data.
Results: The population age was (Mean±SD) 63±9 years (66% female) and diabetes duration was 10±8 years. After the 3-year follow up we recorded no significant changes in BMI but significant improvements (p<0.001) in all groups in systolic (142±17 *vs.* 134±15 mmHg) and diastolic (87±11 *vs.* 80± 9 mmHg) blood pressure, FBG (8.0±2.5 *vs.* 7.2±2.2 mmol/L), HbA1c (7.8±1.5 *vs.* 7.1±0.8%) and total cholesterol (4.7±0.9 *vs.* 4.4±0.7. mmol/L). All these changes were significantly larger in the intervention groups. The percentage of patients at target for all these parameters was significantly (p<0.01) larger in these groups. In the educated groups, we also recorded a significant increment in combined against oral monotheraphy (42 *vs.* 30%) and insulin use (15 *vs.* 9%). Drug consumption and strips for blood glucose represented 64 and 83% of the total care cost at baseline and 3-year follow up, respectively. This cost increased (113%) in the control group while it significantly decreased (11 to 20%) in the intervention groups, particularly in the patient/physician educated group.
Conclusion: Educative interventions implemented at a primary care level to people with T2DM improved the clinical and metabolic outcomes and optimized the use of drugs for DM and other associated cardiovascular risks factors, decreasing the total costs.
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An integrated hospital-community diabetes education network based on self-management school and Telecom

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Background and aims: It was shown from new data that China became the global epicentre of the diabetes epidemic with 92.4 million patients. The challenge for China is to find proper ways to deal with the big problem and help diabetic patients to control the disorder. In the present study, we established an integrated hospital-community diabetes education network based on a self-management school and telecommunication system, and evaluated the efficacy of this network on the metabolic control of diabetic patients in community.
Materials and methods: A total of 524 new diagnosed diabetic patients screened in community were recruited and assigned to intensive group (n=266) or control group (n=258). The patients in intensive group were enrolled in diabetes self-management school located in university hospital first to receive a five sequential days diabetes education course which including diabetes knowledge and diabetes self-management skills delivered by multidisciplinary teacher such as physicians, nurse, dietitian and podiatrist. After graduated from this one week duration school patients were back to community and followed up by community doctor and got subsequent regular advice based on educational focus (information, lifestyle behaviors, glucose monitoring and self-management skills) with telephone, short messages or internet. In the control group, subjects received common education lecturer once a week for up to five weeks followed by regular advice. Outcomes were classified as knowledge, self-management skills and glycemic control in one year using questionnaires and laboratory data.
Results: All patients aged from 9 to 79 years, and average age was 55.19±12.67 years (male 274, female 250), there were no significant difference between two groups in age, gender, diabetes duration, education level, work status, type of insurance, HbA1c, blood pressure, BMI and lipid profile at baseline. After intervention with the integrated diabetes education network, the scores for diabetes knowledge and self-management skills were significantly increased from baseline and higher than control group (P<0.05). Mean HbA1c level was reduced from baseline by 2.43% in intensive group but 1.61% in control group. The percentage of patients with HbA1c<6.5% was significantly higher than control group (68.82% vs 18.53%, P<0.01). The rate of patients' BMI meeting criterion (Male 25kg/m^2,Female 24kg/m^2) was elevated from 54.52% to 61.24% in intensive group(P<0.05). However, there were no significant changes in control group.
Conclusion: Evidence supports the positive effectiveness of the integrated hospital-community diabetes education network on knowledge and self-management skills, finally the better glycemic control was demonstrated.
Supported by: Jiangsu Health Science and Technique Foundation

OP 45 Brain effects on weight regulation and metabolism

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Nesfatin-1-regulated oxytocinergic signalling in the paraventricular nucleus causes anorexia via melanocortin pathway

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Background and aims: Nesfatin-1 is a recently discovered anorectic peptide derived from nucleobindin2. Nesfatin-1 is localized in several brain areas including the hypothalamic paraventricular nucleus (PVN). Starvation decreases NUCB2 mRNA specifically in the PVN. However, the mechanism underlying anorectic action of nesfatin-1 remains unknown. The aim of this study is to clarify the neural pathway through which nesfatin-1 regulates feeding.
Materials and methods: Cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in single neurons were measured with fura-2 combined with immunocytochemical cell identification. The guide cannula was placed stereotaxically into the third ventricle (3V) in Wistar rats or Zucker fatty Rats. Oxytocin (Oxt) releases from PVN slices were measured by radioimmunoassay.
Results: After 3V injection of nesfatin-1, c-FOS was induced in several hypothalamus nuclei including the PVN and in the brain stem nucleus tractus solitarius (NTS). Intra-PVN injection of nesfatin-1 decreased food intake, suggesting that PVN was one of the target sites for nesfatin-1-induced anorexia. 3V nesfatin-1 injection induced c-FOS substantially in the PVN Oxt neurons. In the PVN, nesfatin-1 increased $[Ca^{2+}]_i$ in single neurons immunoreactive to Oxt, nesfatin-1 and both. In the PVN slices, nesfatin-1 stimulated Oxt release. Immunoelectron micrographs revealed nesfatin-1 specifically in the secretory granules of PVN neurons, and immuno-neutralization against endogenous nesfatin-1 suppressed Oxt release in the PVN slices. These results suggested the paracrine and/or autocrine action of nesfatin-1 in the PVN. Nesfatin-1-induced anorexia was suppressed by an Oxt receptor antagonist. Furthermore, Oxt-induced anorexia was abolished by SHU9119, a melanocortin 3/4 receptor (MC3/4R) antagonist, suggesting that MC3/4R is involved in the downstream of nesfatin-1-regulated Oxt neurons. Moreover, Oxt terminals were closely associated with proopiomelanocortin (POMC) neurons in the NTS, and Oxt increased $[Ca^{2+}]_i$ in single POMC neurons in the NTS. In Zucker fatty rats whose leptin receptors are mutated, 3V injection of Oxt induced anorexia that was blocked by SHU9119. The incidence of $[Ca^{2+}]_i$ responses to leptin in NTS POMC neurons was markedly reduced in Zucker fatty rats compared with lean rats. In contrast, the incidence of $[Ca^{2+}]_i$ responses to Oxt in NTS POMC neurons was the same between Zucker fatty and lean rats. This result indicates that Oxt can activate the NTS POMC neurons under leptin-resistant conditions.
Conclusion: Nesfatin-1 activates the activity and secretion of Oxt neurons in the PVN, and Oxt activates POMC neurons in the NTS. This pathway can function independently of leptin signaling and may provide a therapeutic target for treatment of leptin-resistant obese humans showing hyperphagia.
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Role of brain insulin signalling on tissue-specific glucose disposal

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Background and aims: Circulating insulin inhibits hepatic glucose production and stimulates glucose uptake in peripheral tissues. Hypothalamic insulin signaling is required for the inhibitory effects of circulating insulin on endogenous glucose production. In this study, we examined the central effects of circulating insulin on tissue-specific glucose uptake.
Materials and methods: Tolbutamide, an inhibitor of ATP-sensitive potassium channels, was infused in the lateral ventricle (i.c.v.) in hyperinsulinemic euglycemic clamp conditions in chow-fed and in diet-induced obese C57Bl6/J mice. Whole body glucose uptake was measured by D-[^{14}C]glucose kinetics and tissue-specific glucose uptake by 2-deoxy-D-[^{3}H]glucose uptake.
Results: I.c.v. administration of tolbutamide impaired the ability of circulating insulin to inhibit endogenous glucose production by ~20% (P<0.01). Surprisingly, i.c.v. tolbutamide infusion also diminished insulin-stimulated glucose uptake by muscle (-59%; P<0.05), but not by heart or adipose tissue. In contrast, in diet-induced obese mice, high fat feeding abolished the inhibitory effect of i.c.v. tolbutamide on insulin-stimulated glucose production or muscle glucose uptake.
Conclusion: Circulating insulin stimulates glucose uptake in muscle in part through effects via ATP-sensitive potassium channels in the central nervous system, similarly to the effects on hepatic glucose production. In diet-induced obese mice, these effects of circulating insulin via the central nervous system are absent. These observations stress the role of the central effects of circulating insulin in normal physiological conditions and in diet-induced insulin resistance.
Supported by: TI Pharma

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Lipoprotein lipase inhibition in rat hippocampus leads to increase in body weight, fat mass, and basal insulinaemia without change in food intake

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Background and aims: Regulation of energy balance implies peripheral signals (hormones, nutrients) conveying to specialized brain areas. Among them, the hypothalamus and the hindbrain contain both glucose and free fatty acids (FFA) sensitive neurons, which have been demonstrated to be part the central integration of circulating signals of hunger and satiety. However other brain structures associated with the higher-order behavioral response, such as hippocampus, may also participate on the processing of these signals. Hippocampus is densely populated with both ghrelin, leptin and insulin receptors as well as with lipoprotein lipase (Lpl). Thus we postulated that triglycerides hydrolysis by lipoprotein lipase within the hippocampus may play a role in the control of energy homeostasis through local FFA delivery.
Materials and methods: Osmotic minipumps were stereotactically inserted in the hippocampus of male Wistar rats. They received a chronic infusion of tyloxapol (an inhibitor of Lpl activity, 10 µg/day, TYL), or saline (SAL) during 28 days. Food consumption and body weight were measured daily and body composition was analyzed weekly. Between day 20 and day 24, rats were placed in metabolic cages to study metabolic parameters. On day 23, ghrelin orexigenic response (10 nmol IP) was evaluated. On day 27, feeding response to mild hypoglycemia induced by insulin (1 UI/rat) was also analyzed. In another series of experiment, rats were imposed a 24 hour fast. Hippocampus and cortex were collected for the measurement of Lpl activity.
Results: 28 days after minipump implantation, the Lpl activity in hippocampus of TYL rats was decreased by 26% (TYL: 0.91 ± 0.03 U/mg vs. SAL: 1.24 ± 0.08 U/mg, p<0.005) ; TYL rats have gained significantly more weight (TYL: 141.7 ±1.1% vs SAL: 134.6 ±2.8% , p<0.005) than SAL rats while the cumulative food intake stayed unchanged. Increase in fat mass (TYL:+ 20.6 ± 2.4% vs SAL: +9.5 ± 1.5%, p<0.05) accounted for most of body weight gain. This increased fat storage was neither due to a decreased energy expenditure as measured by indirect calorimetry nor to a decrease in total activity. Glycemia, circulating FFA and TG were similar between the two groups, whereas TYL rats displayed basal hyperinsulinemia (TYL: 694.1 ± 42.7pM vs SAL: 485.6 ± 40.6 pM, p<0.05). Food intake measurements after a 24 hour fast or after an insulin challenge were the same in SAL and TYL rats, whereas TYL rats displayed a decreased response to ghrelin (TYL: 5.6 ± 4.6 g/kg vs SAL: 13.9 ± 5.8 g/kg after 4 hours, p<0.05), indicating a potential adaptive mechanism to surfeit food intake and further weight gain.
Conclusion: The inhibition of hippocampal LPL activity by a chronic tyloxapol infusion led to a gain in body weight without any modification of food intake, plasma TG and FFA, and antagonized the orexigenic action of ghrelin. Moreover, tyloxapol-infused rats displayed a hyperinsulinemia without any change in blood glucose, suggesting an autonomic adaptation leading to mild peripheral insulin resistance. Taken together these results support the idea that hippocampal TG hydrolysis might directly influence energy balance regulation at both the metabolic and behavioral levels.

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Hypothalamic leptin improves mitochondrial function in soleus muscle: The role of PI3K signalling

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Background and aims: Complex metabolic diseases such as obesity and type 2 diabetes mellitus (DM2), result from multiple interactions between genetic and environmental factors. DM2 is characterized by insulin resistance in skeletal muscle and other insulin-sensitive tissues, which is accompanied by defective insulin secretion. Muscle insulin resistance is manifested by a reduced capacity of insulin to stimulate glucose uptake due to impaired intracellular signaling. Additionally, insulin-resistant subjects present a reduced capacity to oxidize glucose and lipids in muscle which, at least in part, is due to impaired mitochondrial activity as evidenced by reduction of mitochondrial oxidative and phosphorylative activities. Acting in the hypothalamus, anorexigenic hormones such as leptin and insulin, as well as nutrients can modulate peripheral glucose homeostasis. Some of these effects depend on neural control of hepatic gluconeogenesis. However, the mechanisms by which hypothalamic leptin leads to improved glucose homeostasis in skeletal muscle are incompletely known. The aim of this study was to investigate the molecular mechanism by which ICV leptin improves glucose homeostasis in skeletal muscle.
Materials and methods: Rats were divided in three groups: i) Control (Saline-ICV); ii) Leptin (Leptin-ICV); and, iii) LYL (Ly 294002 + Leptin-ICV). The expressions of PGC1α, Cytochrome c and UCP3 expression was performed by Western Blotting; Citrate synthase activity was analyzed by an espectrophotometric assay and mitochondrial respiration was measured in soleus muscle fibers, permeabilized with saponine, by a high-resolution respirometry assay (OROBORUS). All procedures were approved by the Ethics Committee at the University of Campinas.
Results: The central administration of leptin increased PGC1α expression (>100 %) in skeletal muscle and the ICV administration of Ly 294002 (inhibitor of PI3K) prior to leptin inhibited this effect. Similar effect was observed in Cytochrome c and UCP3 expressions, which increased 480 % and 43 %, respectively, after ICV leptin injection if compared to control group and the previous administration of Ly (ICV) reduced these effects. In addition, leptin injection (ICV) increased the activity of Citrate synthase (80 %) and mitochondrial respiration (18 %) compared to control group.
Conclusion: Leptin injection (ICV) is able to modulate the expression of proteins and mitochondrial function in the skeletal muscle in a hypothalamic PI3K-dependent manner.
Supported by: CNPq / FAPESP

OP 46 Prediction of type 2 diabetes: Can we do better than the usual suspects?

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Low-cost screening model with standard cardiometabolic risk factors for prediction of incident type 2 diabetes: the Whitehall II study

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Background and aims: Accurate prediction of incident type 2 diabetes (T2DM) is pivotal in enhancing strategies for diabetes prevention and cardiovascular risk management. Current strategies generally include a blood sample in a first or second stage, but few studies have systematically compared the accuracy of multiple alternative predictive models. We examined the predictive capability of 5 screening models for incident T2DM in incremental stages of accessibility and cost.
Materials and methods: We tested the following models in a cohort of 4352 men and women aged 39-64 years, free from diabetes (the Whitehall II study): (A) questionnaire only (age; gender; BMI; family history of diabetes; use of antihypertensive/lipid lowering medication); (B) clinical (previous model + blood pressure + waist circumference); (C) low-cost biomarker (previous + fasting glucose + fasting triglycerides + total cholesterol + HDL-cholesterol); (D) medium-cost biomarker (previous + fasting insulin); and (E) high-cost biomarker (previous + ApoA1/B + lipoprotein(a)+ CRP + IL-6 + fibrinogen + von Willebrand factor + factor VII).
Results: Multivariate Cox regression analysis was used to estimate predictive models for 20-year incident T2DM (574 cases). We used a 2-step approach: (i) Receiver Operating Characteristics (ROC) analysis for assessing the improvement in prediction of each subsequent model; and (ii) Based on the results from ROC analysis, backwards elimination analysis for deriving a parsimonious model without reducing predictive performance. The Area Under the ROC Curve (AUC) was higher in all 3 screening models requiring a blood sample (Models C-E) compared to the questionnaire/clinical screening models (Models A and B) (table 1). Compared to model A, the 'clinical model' (B) was not better in predicting incident T2DM (AUC difference=0.001). The 'low-cost biomarker model' (C), improved significantly Model B (AUC difference=0.054). Only marginal improvement was found beyond Model C (addition of fasting insulin and detailed lipid/inflammatory markers) (AUC difference=0.008 and 0.007 respectively). In backwards elimination, we estimated a parsimonious model, which did not reduce the prediction of Model C, containing age, gender, BMI, family history of diabetes, use of antihypertensive/lipid lowering medication, fasting glucose, fasting triglycerides and HDL-cholesterol.
Conclusions: A questionnaire/clinical screening model for predicting incident T2DM is substantially improved by a low-cost blood sample test containing fasting glucose, triglycerides and HDL-cholesterol. Improvements to this model by addition of higher cost biomarkers are not of a clinically relevant magnitude.

Table 1 Area under the ROC curve for additive screening models

Predictive models for incident type 2 diabetes	Area under curve (95% CI)	p for model improvement
Questionnaire only - model A (sex + age + family history of diabetes + use of antihypertensive or lipid lowering medication + BMI)	0.72 (0.69; 0.75)	n/a
Clinical - model B (questionnaire + blood pressure + waist circumference)	0.72 (0.69; 0.75)	0.72
Low-cost biomarker - model C (clinical model + fasting glucose + triglycerides + total cholesterol + HDL-cholesterol)	0.78 (0.75; 0.81)	<0.001
Medium-cost biomarker - model D (low-cost biomarker + fasting insulin)	0.79 (0.76; 0.82)	0.001
High-cost biomarker - model E (medium-cost biomarker + ApoA1/B + Lp(a) + CRP + IL-6 + fibrinogen + vWf + factor VII)	0.79 (0.76; 0.82)	0.015

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The risk of incident diabetes for all possible combinations of metabolic syndrome components

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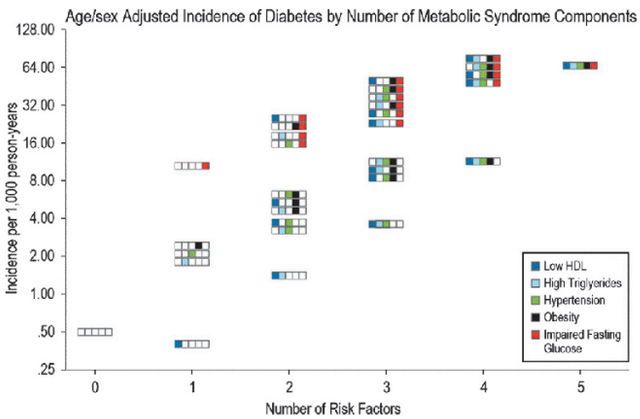
Background and aims: Because metabolic syndrome (MetS) is defined as any three of five criteria, not all persons with MetS have the same cluster of risk factors. Whether the various combinations of criteria confer equal diabetes risk is not known. Our aim was to estimate the risk of incident diabetes simultaneously for all possible combinations of MetS components.
Materials and methods: Using electronic medical record data from a group model HMO, we identified an observational cohort of 58,056 non-pregnant adults age ≥ 30 with no evidence of diabetes and all MetS components measured in 2003-2004. Subjects were followed for up to 5 years for onset of type 2 diabetes. We estimated age and sex-adjusted diabetes incidence for all possible combinations of MetS components.
Results: The overall incidence rate of diabetes was 12.5/1,000 person-years (95% CI 12.1-12.9). The presence of each individual MetS component was associated with significantly greater diabetes incidence than absence of the component. The greatest relative difference was found among those with impaired fasting glucose, with an age and sex-adjusted incidence of 37.4/1000 person years (36.0-38.9) compared to 3.8 (3.6-4.1) among those with normal glucose. Although persons with 1 or 2 MetS factors comprised about 50% of the sample, diabetes occurred in fewer than 5% of these individuals. While the proportion of the sample declined with each additional factor, the proportion developing diabetes increased precipitously with the number of factors present, reaching 28% among those with all 5 components. However, there was wide variation within each count of factors. Depending on which factors were present, incidence varied by >9-fold in patients with 3 risk factors, >5-fold in patients with 4 factors, and >54-fold in patients with <3 factors. Specifically, there was a clear separation between combinations that did and did not include hyperglycemia. In fact, all two-factor combinations that included hyperglycemia had higher incidence rates than three- or four-factor combinations that did not. For example, incidence in patients with only hyperglycemia and obesity was 21.7/1000 person-years (95% CI 17.4-27.1), compared to 11.4 (9.8-13.4) among those with the four component combination of obesity, hypertension, low HDL and elevated triglycerides.
Conclusion: Diabetes risk increases exponentially with MetS factor count, but varies substantially depending upon which factors are present. Hyperglycemia, regardless of the presence of MetS, is a much stronger predictor of incident diabetes than MetS without hyperglycemia.



Supported by: Tethys Bioscience, Inc

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Physiological predictors of changes in glucose tolerance in a non-diabetic population: the RISC Study

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Background and aims: Previous studies using the euglycaemic insulin clamp technique have reported that both insulin resistance and a reduced acute insulin response to intravenous glucose (AIR) predict incident diabetes in isolates (the Pima Indians). We undertook to systematically analyse the relationship between insulin sensitivity/secretion and spontaneous changes in glucose tolerance in non-diabetic subjects.
Materials and methods: In 1,048 subjects from the RISC cohort (561 women and 467 men, mean age 44 years) followed up for 3 years, we measured baseline insulin sensitivity (by a 240 pmol.min.m-2 insulin clamp) and ß-cell function (i.e., fasting insulin secretion rate, total insulin output and ß-cell glucose sensitivity, by mathematical modelling of the C-peptide response to a standard OGTT). Subjects were categorised as NGT, IFG, IGT or T2D and then grouped into stable NGT (if they were NGT both at baseline and follow up, n=809), stable non-NGT (if they were IFG or IGT on both occasions, n=49), progressors (if their glucose tolerance deteriorated, n=129) or regressors (if their glucose tolerance improved, n=61).
Results: In comparison with stable NGTs, progressors, regressors and stable non-NGTs presented a similar clinical (higher prevalence of familial diabetes, older age and higher WHR, fasting and 2-hour plasma glucose, fasting and 2-hour plasma insulin concentrations) and metabolic phenotype (lower insulin sensitivity and reduced ß-cell glucose sensitivity with increased fasting secretion rate and total insulin output). In a multivariate logistic model, both insulin sensitivity and glucose sensitivity were independent negative predictors of progression (odds ratios [95% CI] of 0.70 [0.52-0.93] and 0.42 [0.28-0.65], respectively), while WHR and fasting glucose le-vels were positively associated with progression. The same set of baseline variables also predic-ted regression. At follow up, insulin sensitivity and ß-cell glucose sensitivity were unchanged in the stable NGTs and non-NGTs, declined in the progressors and improved in the regressors.
Conclusion: Among non-diabetic Caucasians, non-NGTs, progressors and regressors appear to derive from a common pool of at-risk subjects, in whom reduced insulin sensitivity and impaired ß-cell glucose sensitivity predict deterioration of glucose tolerance. Changes in both insulin sensitivity and ß-cell glucose sensitivity mark progression as well regression of dysglycaemia.
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Serum fibroblast growth factor 21 and triglycerides independently predict the development of type 2 diabetes

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Background and aims: There is a need to identify novel factors that more accurate predict the risk of developing type 2 diabetes (T2D. Circulating levels of both fibroblast growth factors 19 and 21 (FGF19 and FGF21) show a large interindividual variation in normal humans. Case-control studies have reported correlations between FGF21 and impaired glucose tolerance, insulin resistance, hypertriglyceridemia, obesity and/or T2D. We tested the hypothesis that fasting levels of FGF19, FGF21 or triglycerides could predict the subsequent development of prediabetes (IFG, IGT) or T2D in a healthy cohort followed for a ten-year period.
Materials and methods: In the Stockholm Diabetes Prevention Program (SDPP), a total of 2227 men and 3205 women with normal glucose tolerance were followed-up after ten years. We identified 461 subjects with abnormal glucose regulation (163 with T2DM, 97 of which were newly diagnosed) and compared them with 479 matched controls that remained normal at follow-up. Serum levels of FGF19 and FGF21 were analyzed by ELISA.
Results: At baseline in all subjects (n=940; 396 F and 544 M), mean age was 48.2 yr, BMI 26.3, fasting glucose 4.73 mM, cholesterol 6.23 mM, triglycerides 1.45 mM, FGF19 139 pg/mL, and FGF21 193 pg/mL. At follow-up, subjects with prediabetes or T2D had significantly higher levels of FGF21, 262 and 328, respectively, compared to controls,179; P<0.001 and of triglycerides (1.83 and 2.07 versus 1.33; P<0.001); FGF19 and cholesterol levels were similar, and there were no gender differences. When baseline levels were analyzed in the groups, FGF21 levels were higher in both women and men who later developed prediabetes (213; 228) or T2DM (241; 286) compared to controls (150; 165). This was also seen for triglycerides (prediabetes, F/M, 1.45; 1.86; T2DM, 1.69; 1.89 versus controls 1.11; 1.32); all differences were highly significant (P<0.001). FGF19 or cholesterol did not show any relation to development of glucose intolerance or T2DM. Adjusted for age, BMI, physical activity, smoking, and socioeconomic position, FGF21 and triglyceride level

still predicted an increased risk for glucose intolerance or T2DM (OR for women, 1.91 and 3.63, for men, 2.36 and 4.04, for highest quartile of FGF21; OR for women, 3.33 and 6.82, for men, 3.17 and 2.26 for highest quartile of triglycerides). BMI, FGF21, and triglycerides were shown to be independent as risk predictors when combining women and men in the logistic regression analysis.
Conclusion: Elevated fasting levels of circulating triglycerides and FGF21, but not of FGF19, are associated with a substantially increased risk to develop prediabetes or T2DM in humans. Both elevated FGF21 and triglycerides are independent of each other and of BMI, and point to the presence of an early state of metabolic abnormality predisposing to glucose intolerance. High levels of circulating FGF21 may reflect a state of "resistance" to this newly described metabolic regulator.
Supported by: Swedish Diabetes Association, Research Council, Heart-Lung Foundation

OP 47 Proteomics in diabetes

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Gene-metabolite networks for signature of insulin action in humans
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Background and aims: Insulin is a key metabolic hormone in the body. Few studies describe insulin-dependent transcriptome changes and no data exist for insulin-related plasma metabolome. We investigated the regulation of subcutaneous adipose tissue (SAT) gene expression during different glucose/insulin concentrations and its relation with insulin-induced changes of plasma metabolome.
Materials and methods: Healthy subjects (n=14) underwent a saline-infusion test (INF) and/or a hyperinsulinemic-euglycemic clamp (EC), and/or a hyperinsulinemic-hyperglycemic clamp (HC). SAT biopsy and plasma samples were taken before and at 240 min of the tests. Full human genome Agilent chips were used for transcript profiling, and GC-TOF/MS analysis was applied to get the metabolome of plasma. We created a gene-metabolite network for each experiment and extracted the insulin effects in respect to blood glucose concentrations by alternative network subtraction analysis.
Results: Insulin down regulates about 16% of plasma metabolome under euglycemia, while simultaneous expressional changes are only minor. In the HC experiments, more metabolites were up-regulated. In the subtracted network of EC, insulin was directly connected only with two genes and two metabolites (tetradecanoate and octadecanoate). Moreover, a clock-gene NR1D2 was a major "hub" in this network and was negatively correlated with insulin. The resting network structure included the six hubs of predominantly fatty asides and their derivates. In the network of HC, the major "hub" was fructose. From the six genes, included in this network, five were genes encoding transcriptional factors.
Conclusion: "Gen-metabolite signatures" of insulin action allow us to visualize insulin-dependent patterns of gene-metabolite interactions and help to further understand the mechanisms involved in the pathogenesis of obesity and T2DM.
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Urinary proteome analysis for diagnosis of diabetic nephropathy
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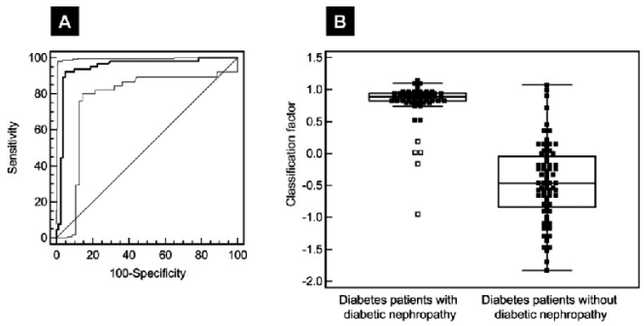
Background and aims: Diabetic nephropathy (DN) is a leading cause of morbidity and mortality in people with diabetes mellitus. Current clinical methods to predict development of diabetic kidney disease are subject to measurement variability and lack predictive power. In several recent studies, it has been shown that urinary proteome analysis enables the definition of biomarkers specific for chronic kidney disease (CKD) in general and for DN; both models includes in the majority different collagen fragments. These might prove valuable in clinical practice, but confirmation of their diagnostic value in independent patient population not included in the original samples used in discovery is required to support their validity and robustness. Therefore, we aimed to validate these biomarkers and biomarker-based models in an independent blinded set of 148 samples, collected prospectively in multiple centers not involved in the original identification of biomarkers to rule out any center-based bias.
Materials and methods: Cases of diabetic nephropathy were defined as albuminuria >300 mg/d and diabetic retinopathy (n=66). Controls were matched for gender and diabetes duration (n=82). High-resolution capillary-electrophoresis coupled to time-of-flight mass-spectrometry (CE-MS) was used to profile the low-molecular-weight proteome in urine of these type 2 diabetic

patients. CE-MS spectra were evaluated employing the previously developed biomarker models for all case and control patients in a blinded setting.
Results: Urinary profiling using CE-MS was successfully applied to urine samples of diabetic patients with or without existing DN. Models for the diagnosis of CKD in general and for the identification of patients with DN in particular, were validated with this multicentre blinded test set and allowed diagnosis of DN with high accuracy (AUC>0.94) (see figure). Furthermore, 61 of the 65 previously identified peptides (94%) were significantly different between these cases and controls.
Conclusion: These data provide the first independent confirmation that profiling of the urinary proteome by CE-MS can adequately identify subjects with DN, supporting the generalizability of this approach. The data further establish urinary collagen fragments as biomarkers for diabetes-induced renal damage that may serve as earlier and more specific biomarkers than the currently used urinary albumin.



Supported by: PREDICTIONS

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Quantitative proteomic analysis of the adipocyte plasma membrane proteome identifies the Sodium/Hydrogen exchanger NHE6 as a novel insulin-responsive protein

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Background and aims: In mammals the maintenance of glucose homeostasis is achieved via insulin-stimulated translocation of the glucose transporter GLUT4 to the plasma membrane (PM) of adipocytes and myotubes. After insulin stimulation the PM is the target membrane for intracellular vesicles containing GLUT4 and so the proteins present there both before and after insulin stimulation along with their phosphorylation status are of great interest. In order to identify novel regulators of insulin-stimulated GLUT4 translocation to the PM, we conducted a quantitative and comprehensive proteomic screen of this organelle, before and after insulin stimulation. We have also taken advantage of the high binding affinity between many of the substrates of Akt, a key regulator of insulin stimulated GSV translocation, and 14-3-3, by using 14-3-3 affinity chromatography to identify novel insulin-regulated phospho-proteins acting downstream of this kinase at the PM.
Materials and methods: Stable isotopes were incorporated into mouse 3T3-L1 adipocytes and protein abundance and 14-3-3 binding in the PM from unstimulated cells, cells treated with 100 nM insulin for 20 minutes and cells treated with insulin plus the PI3Kinase inhibitor wortmannin was quantitated by LC-MS/MS and immunoblotting. PM fractions were isolated using cationic colloidal silica and these were further fractionated by a high salt, high pH wash. 14-3-3 binding proteins were identified using human 14-3-3-beta coupled to cyanogen bromide activated Sepharose 4B and all LC-MS/MS data were processed, searched and quantified using the Maxquant software version 1.0.13.13 package.
Results: These studies revealed 35 proteins that underwent insulin-dependent translocation to the PM and included both known (GLUT4, IRAP, Transferrin receptor protein-1, Cation-dependent mannose-6-phosphate receptor and Syntaxins -6 and -12) and previously unknown insulin-responsive proteins. An additional 9 insulin-responsive proteins, including cGMP-inhibited 3',5'-cyclic phosphodiesterase B were identified by 14-3-3 pull-down. More detailed studies using a Sodium/hydrogen exchanger memeber 6 (NHE6) specific antibody showed that this protein underwent a 2-3 fold increase at the PM and is expressed predominantly in brain and adipose tissue. Furthermore, in 3T3-L1 fibroblasts, NHE6 protein expression is up-regulated during the differentiation process and is partially co-localised with GLUT4.
Conclusion: The approach demonstrated here has led to the most extensive characterisation of a mammalian PM proteome and its constituent insulin-responsive compartments. Differential analysis identified 27 novel insulin-responsive proteins, including the sodium/hydrogen exchanger NHE6. Insulin-stimulated NHE6 translocation may explain the observed elevation in intracellular pH induced by this hormone and thus NHE6 may play an important role in insulin action.
Supported by: NHMRC and DART

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Urinary collagen fragments are significantly altered in diabetes: a link to pathophysiology

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Background and aims: Although all forms of diabetes mellitus (DM) are characterized by hyperglycemia and β-cell dysfunction, the pathogenesis of DM is variable, comprising different degrees of β-cell dysfunction, apoptosis, inflammation and immune responses. Proteome analysis holds the promise of delivering substantial insight into the pathophysiological changes associated with different types of diabetes. Recently, we identified and validated urinary proteomics biomarkers for diabetes, and diabetes-associated micro- and macrovascular complications. Based on these initial findings, we aimed to further validate urinary proteomics biomarkers specific for diabetes in general, and specifically associated with either type 1 (T1D) or type 2 diabetes (T2D).
Materials and methods: The low-molecular-weight urinary proteome of 902 subjects from 9 different clinical centres, 315 controls and 587 patients with T1D (n=299) or T2D (n=288), was analyzed using capillary-electrophoresis mass-spectrometry.
Results: A previously discovered panel of 261 urinary biomarkers (102 were sequenced) based on 205 subjects distinguished DM subjects from control subjects with 94% (95% CI: 92-95) accuracy in 697 independent subjects. To identify biomarkers that differentiate T1D from T2D, a subset of normoalbuminuric patients with T1D (n=68) and T2D (n=42) was employed, enabling tentative identification of 204 biomarker candidates (68 were sequenced) differentially regulated between T1D and T2D. These biomarkers distinguished T1D from T2D in an independent validation set of normoalbuminuric patients (n=108) with 93% (95% CI: 86-97%) accuracy. When applied to patients with impaired renal function (n=369) accuracy was 91% (95% CI: 88-94%). Most of the biomarkers significantly associated with diabetes, and those that are apparently diabetes-type specific, were specific collagen fragments, indicating highly significant changes in collagen turnover and extracellular matrix as one hallmark of the molecular pathophysiology of diabetes. Additional indications for chronically sustained renal injury mediated by inflammatory processes and pro-thrombotic alterations were observed.
Conclusion: These findings, based on the largest proteomic study ever performed on subjects with DM, pinpoint potential differences in the pathophysiology of T1D and T2D that may improve understanding of diabetes and diabetes-associated complications and result in improved therapeutic strategies.
Supported by: PREDICTIONS, InGeniousHyperCare

OP 48 Biomarkers of type 1 diabetes

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Age-related and islet autoimmunity associated differences in metabolites of the amino acid and lipid metabolism in children at high risk for type 1 diabetes

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Background and aims: Data from the BABYDIAB study demonstrate that islet autoimmunity in children of mothers or fathers with type 1 diabetes (T1D) is initiated early in life with two phenotypically distinct peaks of antibody incidence before age 4 and after age 7 years. Changes in lipid and amino acid metabolism are suggested to precede the development of T1D. The aim of this study was to examine whether there are changes in serum metabolite profiles (metabolomics) which are characteristic for early (< age 4 years) compared to late (> age 7 years) initiation of islet autoimmunity.
Material and methods: Metabolites of the amino acid and lipid metabolism were measured in samples from 70 BABYDIAB children, including 13 who developed islet autoantibodies (AA) early (< age 4 years) and 22 who developed islet AA late (> age 7 years), and 35 age, date of birth, and HLA-matched children who remained islet AA negative ('controls'). Metabolites and lipids were measured quantitatively in the first antibody positive serum sample or in aged-matched samples of 'controls' using UPLC coupled with UV detection and UPLC-MS, respectively. The 511 detected molecular lipids were clustered into 12 groups (LC). Concentrations were compared using the Mann-Whitney-U-Test.
Results: Specific changes in metabolite and lipid profiles were identified in BABYDIAB children. These included both age-related and antibody appearance related. Regardless of antibody status, children aged >7years had higher concentrations of glutamine (p=0.004), arginine (p=0.008), glycine (p<0.0001), and citric acid (p=0.006) as compared to children <4years of age. Similarly, older children had higher concentrations of LC 2 and 4 which represent proinflammatory lysophosphatidylcholines and sphingomyelins compared to younger children (p<0.0001 and p=0.002), respectively, whereas younger children had higher concentrations of lipids in LC10, containing saturated triglycerides, as compared to older children (p<0.0001). Related to the appearance of islet AA we found lower concentrations of methionine (p<0.0001), ethanolamine (p=0.02) and glutamic acid (p=0.03) in children who developed islet AA early, and lower concentrations of hydroxyproline in children who developed islet AA late as compared to islet antibody-negative children of the respective age-group (p=0.03). Furthermore for both age groups (early and late) we found higher concentrations of LC8, a functionally diverse lipid cluster of specific phospholipids and triglycerides, in children developing islet AA compared to children who remained islet antibody-negative (p=0.0001).
Conclusion: These data demonstrate that there are changes in metabolomic profiles that appear specifically associated with the appearance of islet autoantibodies and with the age of antibody seroconversion. These changes may reveal important novel pathways related to the pathogenesis of autoimmune diabetes. Additional marked age related changes of metabolomic profile which are independent of autoimmunity indicate that age is an important confounder for any future studies using metabolomics technology.
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A distinct metabolic profile at birth identifies children developing type 1 diabetes before 20 years of age

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Background and aims: Islet autoantibodies are early markers of developing type 1 diabetes and are useful for disease prediction. Recent data suggests that metabolomics analysis may uncover metabolic disturbances prior to seroconversion and clinical diabetes, and that specific alterations may already be detectable at birth. The aim of this study was to confirm evidence of altered metabolic pattern at birth in children who developed type 1 diabetes before 20 years of age.
Materials and methods: Unbiased metabolomics analysis was applied in a case-control analysis of paired cord blood samples from 24 children diagnosed with type 1 diabetes at a median age of 17 years (range 3.2-18.8) and an equal number of healthy controls matched for age (year/month/day) and gender. Cord blood serum from the cases and controls was collected in 1970-1991. All samples were stored frozen at -20°C and had been subjected to an equal number of freeze-thaw cycles. Children born to diabetes mothers were excluded. The analysis of coded samples was performed randomly by Gas Chromatography/Time-of-Flight Mass Spectrometer (Pegasus 4D; Leco). The potential effect of long-term storage on metabolites was investigated through Spearman´s *rho* correlation between metabolite concentrations and sample age. Paired case-control differences were calculated with nonparametric Wilcoxon rank-sum test ($p<0.05$ considered significant).
Results: The type 1 diabetes progression related changes were detected in specific groups of metabolites. The cord blood serum metabolome from the children who develop type 1 diabetes showed 1.2-1.4 -fold higher concentrations of several fatty acids compared to the controls: linoleic acid $p<0.008$, myristic acid $p<0.002$, lauric acid $p<0.002$, stearic acid $p<0.017$, palmitoleic acids $p<0.02$. Oleic and palmitic acid did not differ between cases and controls. Fatty acids did not correlate with the years of storage except for lauric acid that increased up to 2-fold in the oldest samples ($p<0.001$) in cases as well as in controls. There was no significant correlation between metabolite concentrations and age at diagnosis or maternal age in the case group.
Conclusion: Although storage time and conditions are critical in metabolomics studies for the potential confounding effects of lipolysis and proteolysis on the metabolites, our samples from carefully matched cases and controls enabled the use of older samples. In our study the metabolite pattern at birth distinguished children who developed type 1 diabetes from the matched control children who remained healthy. The evidence of these early metabolic alterations may give new insights into type 1 diabetes early pathogenesis and identify potential triggering factor of islet autoimmunity possibly involving gestational events. The identification and validation of key metabolites marking the progression to islet autoimmunity and clinical onset may also provide new markers for disease prediction.
Supported by: DIAPREPP

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GAD65- and (pro)insulin-specific CD4+ T cells detected by MHC class II tetramers in diabetes-associated autoimmunity

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Background and aims: Autoreactive CD4+ T cells contribute to the destruction of insulin producing beta-cells in type 1 diabetes (T1D). The aim of this study was to investigate the frequency of circulating GAD65-, proinsulin- and insulin-specific CD4+ T cells during the pre-clinical phase of T1D and to evaluate the naïve/memory phenotype of these autoreactive T cells.
Materials and methods: Using MHC class II tetramers, we have analysed the frequency of GAD65- (274-286 and 555-567 557I), proinsulin- (B24-36) and insulin- (A1-15 and A6-21) specific CD4+ T cells in 26 children with recently diagnosed T1D, 48 multiple autoantibody-positive children and 70 HLA- and age-matched control children. In a smaller group of children memory and naïve T-cell responses to the same autoantigens were investigated as well.
Results: We observed that the children with multiple autoantibodies recognize the GAD65 555-567 (557I) peptide more frequently (52.4%) than the children with T1D (22.2%) or controls (30.5%) (P=0.027). Furthermore, multiple autoantibody-positive children had more often memory (CD45RO+) T cells specific for GAD65 555-567 compared to controls (P=0.028), in whom none (n=27) displayed a positive memory T-cell response to this peptide. Interestingly, in children with T1D GAD65 555-567 specific T cells were both memory and naïve, (P=0.029 and 0.044, in comparison to controls, respectively). The other investigated peptides were frequently recognized in the study population, but no statistically significant differences were observed.
Conclusion: These results indicate a higher CD4+ T-cell reactivity to the GAD65 555-567 epitope in children with recently diagnosed T1D and in multiple autoantibody-positive children compared to unaffected controls testing negative for autoantibodies.

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Novel triples mix radio binding assay for the ZnT8 (ZnT8RWQ) autoantibody variants in children with newly diagnosed diabetes

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Background and aims: Autoantibodies (A) against the ZnT8 transporter are common in type 1 diabetes. The ZnT8A analyses are complicated by the fact that it is not only one but three autoantigens representing ZnT8R (arginine), ZnT8W (tryptophan) and ZnT8Q (glutamin) at amino acid position 325. It is important to detect all ZnT8A variants not only to predict type 1 diabetes but also to select subjects for prevention and intervention clinical trials. The aims were: 1) to develop an autoantigen triple mix Radio-Binding Assay (RBA) to screen for ZnT8A; 2) to identify the individual ZnT8-RWQA reactivity and 3) to validate the triple mix ZnT8Ab RBA in children with newly diagnosed type 1 diabetes.
Materials and methods: Serum or plasma samples were obtained from 1868 patients in the on-going Better Diabetes Diagnosis (BDD) study. BDD is a nationwide prospective cohort study that involves newly diagnosed diabetes children who are <18 years from 40 (95%) pediatric clinics in Sweden. The cDNA coding for the C-terminal end of each variant was subcloned into the high efficiency *in vitro* transcription translation pTNT vector (Promega, Madison, WI, USA) following site-directed mutagenesis. The ZnT8 variants were labelled with 35S-methionine and used at 425 cpm/μL in standard RBA separating free from autoantibody-bound autoantigen with Protein A-Sepharose. All samples were first analyzed for autoantibodies to each individual variant of ZnT8. Bound radioactivity was converted into in-house units using a standard curve generated negative controls and by a type 1 diabetes serum standard with high reactivity for each of the ZnT8R, ZnT8W and ZnT8Q antigens. All samples were also analyzed in a triple mix RBA to detect all three variants (ZnT8-RWQA) simultaneously. The ZnT8-RWQA RBA was performed after the three variants were mixed at a final concentration of 425±25 cpm/μL. The bound radioactivity were converted into in-house units using negative controls and a type 1 diabetes serum standard with high reactivity for all three ZnT8A variants.
Results: We examined sera from 1868 (53% males) newly diagnosed incident type 1 diabetes patients <18 years who were registered in the BDD study. ZnT8-RA was detected in 964 (52%) patients, ZnT8-WA in 895 (48%) and ZnT8-QA in 609 (33%). Autoantibodies to all three variants were found in 527 (28%) patients. ZnT8-RWQA was detected in 1267 (68%) patients representing only 5.6% false positive samples. None of the new-onset patients was false negative for ZnT8-RWQA. The highest ZnT8A frequencies were found among the 5-9 (67%) and 10-14 (72%) year old patients. Neither the ZnT8-RWQA nor the three individual variants showed gender variation.
Conclusion: The major finding in this study was that the ZnT8A triple mix assay had a low false positive rate and a negligible false negative rate. The ZnT8A triple mix assay would therefore be highly suitable for screening the general population, as it is likely not to miss individuals who have anyone of the three variants.
Supported by: DIAPREPP, NIH, SCDF, SRC, SDARF, SCCFRD and SKL

PS 1 Monogenic forms of diabetes

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Differences in thrombotic and inflammatory markers comparing subjects with maturity onset diabetes of the young and type 2 diabetes

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Background and aims: Mutations in the Hepatocyte Nuclear Factor 1α (*HNF1A*) gene are the commonest cause of Maturity onset diabetes of the young (MODY) in the UK, characterised by β-cell dysfunction without insulin resistance. Cardiovascular disease is the main cause of mortality in individuals with diabetes and it has been demonstrated that fibrin network structure and its resistance to lysis determines predisposition to atherothrombotic disease. The aim of this work was to investigate clot structure/fibrinolysis and cardiovascular inflammatory markers in subjects with HNF1A-MODY and type 2 diabetes (T2DM).
Materials and methods: Subjects with HNF1A-MODY (n=18, mean age 35.3 years, 11 female) and T2DM (n=12, mean age 43.3 years, 8 female) were recruited. Fibrin clot structure was measured using a validated turbidimetric assay; clot final turbidity, a measure of clot density and time from full clot formation to 50% lysis, an indicator of lysis potential, were recorded. Plasma fibrinogen, plasminogen activator inhibitor-1 (PAI-1) and the cardiovascular inflammatory proteins complement-3 (C3) and C-reactive protein (CRP) were also measured by ELISA. All values are given mean±SEM.
Results: The groups did not significantly differ in age, gender, insulin use, blood pressure, HbA1c or BMI. The HNF1A-MODY group recorded lower final turbidity compared with T2DM (0.33±0.13 vs 0.44±0.12 au respectively, p=0.03). Time from full clot formation to 50% lysis was shorter in the HNF1A-MODY group than T2DM (473±159 vs 588±199s, p=0.048). Despite differences in final turbidity and lysis time, fibrinogen levels were similar (2.8±0.7 vs 2.9±0.5g/L, p=0.6), as were PAI-1 levels (1.99±1.27 vs 1.65±0.82ng/ml, p=0.47). C3 levels were significantly lower in HNF1A-MODY subjects than T2DM (0.58±0.20 vs 0.80±0.21mg/ml, p=0.006), whereas CRP was not different (1.16±2.07 vs 1.85±1.09 mg/L, p=0.3). C3, a protein known to be incorporated into the fibrin network, correlated strongly with final turbidity (r= 0.66, <0.001) and time to 50% lysis (r=0.7, p<0.001).
Conclusion: Our data suggest that, despite phenotypic similarities between the groups, those with T2DM have tighter clot structure, which is more resistant to fibrinolysis compared with HNF1A-MODY. The similar plasma levels of fibrinogen and PAI-1 suggests this is due to post-translational modifications in the fibrinogen molecule or other, yet unidentified, plasma factors. One possible explanation may be related to higher C3 plasma levels in T2DM patients, directly affecting clot structure and lysis time. Taken together, our data suggest that patients with HNF1A-MODY are at lower risk of macrovascular diabetic complications than matched T2DM patients.
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A large part of the MODY patients in Slovakia do not have any mutation in GCK, HNF1A, HNF4A, HNF1B, KCNJ11 or insulin genes

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Introduction: Maturity Onset Diabetes of the Young (MODY) is a heterogenous group of monogenic diabetes with early onset, familiar appearance, and autosomal dominant inheritance. MODY could be caused by a mutation in one of the nine MODY genes, or genes causing predominantly neonatal diabetes. Nevertheless, large number of MODY families does not have any mutation in the genes so far identified. Patients with clear phenotype of MODY diabetes without one of the known mutation, are thus labelled as MODY-X subjects.

Aim: Of this study was to identify the genetic background of families, fulfilling the MODY clinical criteria, and to calculate the frequency of selected MODY subtypes and MODY-X in Slovakia.
Methods: 239 patients from 97 families fulfilling the MODY clinical diagnostic criteria (Ellard et al, 2008), were actively identified in the diabetes outpatient clinics throughout Slovakia. The relevant genes responsible for MODY (*GCK, HNF1A, HNF1B, HNF4A, KCNJ11* and *insulin*) were analyzed using the direct sequencing technique and MLPA.
Results: 152 patients from 57 families had 35 different mutations in one of the target genes: 33 probands and 65 family relatives had a mutation in *GCK* gene; 22 probands and 29 their family relatives had a mutation in *HNF1A* gene; one family (2 pts) had a mutation in the *HNF4A* gene, and one proband had a *HNF1B* whole gene deletion. No *KCNJ11* and *insulin* gene mutation carriers were found.
Conclusion: Out of 97 families, 40 families had no mutation in the genes analyzed. Among the MODY subtypes, the most prevalent are the *GCK* mutations (34% of MODY), followed by *HNF1A* (23%), *HNF4A* (1%) and *HNF1B* (1%) mutations. Despite of DNA analysis of the six MODY genes, the MODY-X families account up to 41% of all cases. The latter is a great challenge for identification of further genes leading to MODY diabetes.
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Liver adenomatosis in MODY 3 diabetes mellitus families: screening 174 patients
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Background and aims: Liver adenomatosis is a rare disorder susceptible to hemorrhagic complication and rarely malignant transformation. Liver adenomatosis results from the biallelic inactivation of the HNF1A gene which encodes a transcriptional factor.The epidemiology of the disease is poorly documented. The aim of this study was to evaluate the frequency of liver adenomatosis in a population of MODY 3 patients carrying a HNF1A germinal mutation, and to describe the clinical course of the disease in the patients affected by the liver disease.
Materials and methods: 174 patients from 74 families MODY 3 were included in 13 French centres. Screening for liver adenomatosis was performed by systematic liver ultrasonography. When the disease was suspected, liver CT or MRI was performed for confirmation of the diagnosis. Histopathological analysis was performed when a surgery was mandatory.
Results: Among 137 patients carrying an HNF1A mutation, 9 cases of liver adenomatosis were identified in 7 different families. Mutations were spread all over the coding region of the HNF1A gene. Patients mean age was 32,8 years (11-56), the M/F sex ratio was 2/7, 7/9 patients had diabetes mellitus, the two remaining patien ts were children presently normoglycemic. Liver imaging showed adenomas of various sizes from less than ten mm (1/9), to larger lesions (8/9) up to 120 mm. Liver biology was near normal in all patients. One case of adenomatosis was revealed by two episodes of internal hemorrhage. Five women had ten pregnancies without any complication nor progression of the adenoma size. Histopathological confirmation of liver adenomatosis was available in five patients, and all adenomas were steatotic at variable degree.
Conclusion: The frequency of liver adenomatosis in this cohort of MODY 3 diabetic patients (6,5%) and the risk for hemorrhagic complication of the disease favours the systematic screening for liver adenomatosis in MODY 3 families.

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Hypomagnesaemia revealing Maturity Onset Diabetes of the Young (MODY) type 5 linked to TCF2 mutation
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Two to 5% of non insulin dependent diabetes (NIDD) are related to ß cell genetic anomalies among which six types of Maturity Onset Diabetes of the Young (MODY), linked respectively to mutations of genes encoding 1) HNF-4alpha, 2) GCK, 3) HNF-1alpha, 4) IPF-1, 5) the transcription factor HNF-1ß (encoded by *TCF2*) and 6) NeuroD1/ß2. A 34 year-old woman was referred for uneasiness with tremor and paresthesia. Hypertension was present in her two parents. Her own medical history was marked by 4 episodes of seizures at 8 months one of them related to hypocalcemia (60mg/l; Normal range (N): 95-105) with otherwise negative etiological investigations except fever in another episode. She developed mild mental retardation without dysmorphic features, and was regularly menstruated. NIDD was discovered at 18 years old. At 34 years old, her BMI was 25 and her blood pressure 130/80 mmHg with normal clinical examination. Biological investigations without treatment showed severe hypomagnesemia (12 mg/l; N: 18-20) by renal wasting (152 mg/24h; N: 80-180), with calciuria between 72 and 130 mg/24h; N:100-250) with normal blood creatinine (10mg/l), potassium (4.1mmol/l), calcium (96 mg/l), HCO3- (28 mmol/l), PTH (34 pg/ml), 25 OH vitamin D (50 nmol/l; N:25-150), renin and aldosterone levels. There was nor proteinuria, microalbuminuria, neither sediment anomalies. The NIDD was characterized by normal blood C-peptide level (2.2 ng/ml (N: 0.5-2, neutral HLAdq susceptibility for type 1 diabetes and undetectable islet auto-antibodies. HbA1c was 7.2% (N<6.5). Intravenous glucose tolerance test performed with and without intravenous magnesium repletion, showed high insulinemia when blood magnesium levels were low, arguing for severe insulin resistance (insulinemia 200 to 250 mU/l), corrected by magnesium repletion (insulinemia 10 to 15 mUI/l) for similar blood glucose levels (reaching 3,5g/l). Kidney cysts, hypertension and increased liver enzyme levels (TGO 109, TGP 218; n<40 UI/l) occurred with time. Genetic investigation showed a heterozygous deletion of the 9 exons of *TCF2* gene, which was identified nor in 2 of her 3 living brothers and sisters (one was dead). To conclude, mild low magnesium level has been reported in NIDD. Nevertheless the severity of hypomagnesemia linked to renal wasting suggested a tubulopathy. Early occurrence of hypomagnesemia with hypocalcemia was compatible with *TRPM6* mutations, whereas isolated hypomagnesemia are associated with mutations of *pro-EGF* (with often mental retardation) or gamma sub-unit of Na-K ATPase. Finally, genetically determined diabetes with hypomagnesemia can be linked to mitochondrial cytopathy or *TCF2* mutations. The presence of renal cysts suggested this last diagnosis, even if in MODY 5, hypomagnesemia is rarely so severe. In this situation low magnesium levels are probably explained by the regulation of FXYD2 transcription, which participates to magnesium tubular reabsorption, by HNF 1ß.

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Genetic counselling for MODY, MIDD and other type of diabetes mellitus
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Background and aims: Recently, genetic testing is provided in the clinical practice of diabetes mellitus. We have started the genetic counseling for diabetes since May 2006, however, such medical service is still few and no report has been published in Japan. The aim of this study is to clarify the current status of genetic testing for diabetes and evaluate the quality of genetic counseling by a hospital based setting.
Materials and methods: We reviewed the medical records of the cases who received genetic counseling for diabetes in the Institute of Medical Genetics at Tokyo Women's Medical University from May 2006 to March 2010. The number of such proband was 22. After informed consent was obtained from the patients, the screening for mutations in the candidate genes had done at the Diabetes Center and other academic institute according to the disease.

Results: Two patients were self referral and 20 were referred by other physicians. Ten patients had been diagnosed with mitochondrial diabetes (MIDD) including mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke (MELAS) syndrome, 8 patients with maturity-onset diabetes of the young (MODY), one with early onset type 2 diabetes having renal hypoplasia, one with diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD) or Wolfram syndrome, one with type 2 diabetes and cardiac myopathy, and one with just type 2 diabetes. We found 4 cases with mutations in the HNF-1a/MODY3 gene among 7 cases of MODY (57%) who received the genetic testing; two cases with P291fsinsC, one with R200W and one with P379S (CCT>TCT). A subject with renal hypoplasia but lacking family history of diabetes showed a large deletion at chromosome 17q12 including HNF-1b/MODY5 gene. Among 8 cases with MIDD, 6 cases were positive for 3243 A>G mutation in the mitochondrial gene. Subjects with DIDMOAD had found to have compound heterozygous mutation in the exon 8 of wolfram 1 gene (L468X, del509VYLLY). Genetic testing for three patients with MIDD/MELAS and one with MODY had been carried out at other institutions. They had not received appropriate counseling regarding the nature of their condition and visited our clinic to obtain more information regarding the results of the genetic testing.
Conclusion: Our study indicates that 1) a need of the genetic counseling for diabetes exists in Japan, 2) MODY3 is prevalent among MODY in our institute, 3) patients should receive genetic counseling and psychological evaluation before any genetic testing are carried out.
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Insulin sensitivity in children with permanent neonatal diabetes mellitus treated with sulfonylurea
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Background and aims: Adults with permanent neonatal diabetes mellitus (PNDM), carriers of a Kir6.2 mutation were characterized by decreased insulin sensitivity. The transfer to sulphonylurea therapy improved insulin sensitivity in them. Aim of the study was to estimate insulin sensitivity in PNDM children with Kir6.2 mutation during insulin therapy and after their transfer to sulfonylurea.
Materials and methods: Three children aged 9, 11, 14 years with diabetes mellitus recognized in the first 6 months of life with confirmed Kir6.2-activating mutations were included into the study. Euglycemic-hyperinsulinemic clamp was performed to estimate insulin sensitivity. Glucose disposal rate (M value) determined during the last 30 min of the test was calculated as a surrogate of insulin resistance. The height, weight were measured and body mass index (BMI) was calculated. HbA1c was measured by HPLC. All examinations were performed on insulin therapy and 6 months after transfer to sulphonylurea.
Results: Baseline M values in children were: 15,0; 7,25 and 13,33 mg · kg^{-1} · min^{-1}, (mean 11,86±4,08). The mean BMI was 15,27±1,10 kg· m^{-2} and HbA1c was 6,93±0,38%. Six months after the initiation of sulfonylurea therapy we did not observe significant reduction of body weight (Δ BMI = - 0,46±0,54; p=0,28). The improvement in metabolic control was noted (Δ HbA1c = - 1,03±0,25%; p=0,02). A substantial improvement in insulin sensitivity was found in all examined patients. Mean decrease in M value (Δ M) was 2,92±1,88 mg · kg^{-1} · min^{-1} (p=0,11). After adjusting to BMI and HbA1c Δ M value was -1,21±1,52 mg · kg^{-1} · min^{-1} (p=0,33).
Conclusion: In PNDM children insulin resistance was not observed during insulin therapy. A slight improvement of insulin sensitivity was noted after initiation of sulphonylurea therapy, but it was influenced by improvement of metabolic control.

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The first case report of sulphonylurea use in a woman with PNDM due to KCNJ11 mutation during a high risk pregnancy
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Background and aims: Sulphonylureas (SU) were proven to be more effective than insulin in most KCNJ11 gene (encoding Kir6.2) related Permanent Neonatal Diabetes Mellitus (PNDM) patients. So far, there was no data on SU use in pregnancy in a KCNJ11 mutation carrier. Here, for the first time, we report SU use in a woman with PNDM due to the KCNJ11 mutation during a high-risk pregnancy.
Materials and methods: A woman with the R201H Kir6.2 mutation became pregnant at the age of 37. The patient had been on glipizide 30 mg for 3 years; her HbA1c level was 5.8%. She was diagnosed with chronic diabetes complications and a congenital defect of urogenitary tract- a bicornuate uterus with septum. As the effect of SU on fetal development is uncertain, she was switched to insulin after the pregnancy diagnosis, however, the subsequent glycemic control was unsatisfactory, with episodes of hyper- and hypoglycemia. Thus, in the 2nd trimester, the patient was transferred to SU (glibenclamide 40 mg).
Results: Transfer to glibenclamide resulted in stabilization of glycemic control; HbA1c in the 3rd trimester was 5.8%. The prenatal genetic testing excluded the Kir6.2 R201H mutation in the fetus. The preterm Cesarian delivery was carried out in the 35th week due to cardiotocography abnormalities. The Apgar score of the newborn boy (weight 3010g, 75th percentile) was 8 at 1 min. He presented with hypoglycemia, transient tachypnea of the newborn (TTN), and hyperbilirubinemia. The recovery was uneventful. No birth defects were recorded. His development at the 9th month of life was normal.
Conclusion: We show a high-risk pregnancy in long-term PNDM that in spite of perinatal complications ended with the birth of a healthy child. SU, that seem to constitute an alternative to insulin during pregnancy in Kir6.2 related PNDM, were used during the conception period and most of the 2nd and 3rd trimester. Prenatal molecular testing should be considered in pregnant women with PNDM especially when other medical indications for amniocentesis, like older age of the mother, are present.
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Diabetes onset among carriers of the WFS1 gene mutation in the families with Wolfram syndrome
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Background and aims: Wolfram syndrome is a rare form of diabetes mellitus associated with an optic atrophy and disorders of different organs e.g. deafness, endocrinologic, neurologic and hematologic abnormalities. This syndrome is caused by the mutations in wolframin gene localized on chromosome 4p16.1 (*WFS1*). Wolfram syndrome is inherited as an autosomal recessive trait. The aim of the study was an evaluation of the clinical course of this syndrome and the mutations in the *WFS1* gene in the Polish patients.
Materials and methods: 9 patients with the clinical symptoms suggesting Wolfram syndrome (at least diabetes mellitus and optic atrophy) aged: 10-24, mean 15.4±4.9 years and 22 first-degree relatives aged: 3-50, mean 27.8±15.7 years were examined. The genetic analysis was carried out by direct DNA sequencing of *WFS1* gene. Additionally, a cosanquinity evaluation using the multiplex-PCR method at16 polymorphic loci was performed.
Results: In 9 patients with Wolfram syndrome nine mutations in *WFS1* gene were identified. In the patients the mean age of diagnosis of diabetes mellitus was 5.6±1.8 years. Six patients were homozygous for the following mutations: V412fs, S443R, W539X, V659fs. They developed diabetes at the age of 5, 5, 4, 4, 5 and 8 years, respectively. Three patients were complex heterozygous for the following mutations: S167fs, Q392X, Y513fs, W648X, V779G. They

developed diabetes at age of 8.8, 7 and 3.8 years, respectively. Among different nine mutations five - S167del, S443R, Q392X, Y513fs, W539X were novel. Among first-degree relatives 17 individuals were heterozygotic carriers of the mutation. In this group no clinical symptoms characteristic for Wolfram syndrome were noticed and none of these carriers had diabetes (age at examination: 34.2±16.1 yrs).
Conclusion: Mean age of diagnosis of diabetes among the Polish patients was typical for Wolfram syndrome, however, complex heterozygous patients were slightly older at diabetes onset. Interestingly, none of the heterozygotic carriers of *WFS1* mutation suffered from diabetes, which is in contrary with the results from the whole genome association studies suggesting that some common SNPs in *WFS1* may predispose to type 2 diabetes.
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PS 2 Genetics of type 1 diabetes

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Clinical and genetical characterisation of a series of patients with type 1 diabetes induced by interferon therapy
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Background and aims: Interferon alpha is widely used for treatment of several diseases including chronic hepatitis C, and rarely causes type 1 diabetes. The aim of this study is to clarify clinical and genetical characteristics of interferon-induced type 1 diabetes.
Materials and methods: Subjects were consecutive 12 patients in whom type 1 diabetes occurred after interferon therapy due to chronic hepatitis C during 1998-2009. To compare clinical pictures, 128 patients with type 1A diabetes who had at least one data about anti-GAD65 antibodies or serum fasting C-peptide levels during first 4 years were enrolled. As genetic controls, 10 patients in whom diabetes did not develop after interferon therapy due to chronic hepatitis C were enrolled. In addition, 136 normal control subjects were enrolled. GAD antibodies were assayed by radioligand binding assay, and C-peptide was measured by sensitive radioimmunoassay. HLA-A, -DRB, -DQA and -DQB alleles were typed by PCR-RFLP methods.
Results: Ten of 12 (83.3%) patients with interferon-induced type 1 diabetes showed ketosis at the onset and 11 of 12 (91.7%) needed insulin therapy within 3 months after the onset of diabetes. Titer of GAD antibodies were higher in the patients with interferon-induced type 1 diabetes than those with type 1A diabetes at the onset [median (range): 3,309 (15-110,000) vs. 7.7 (<1.2-38,000) U/ml, p<0.0001], at 1year [347 (31-10,000) vs. 1.6 (<1.2-3,194) U/ml, p=0.0041], and at 2-4 years [1,248 (37.6-6,379) vs. 3.5 (<1.2-6,514) U/ml, p=0.0002] after the onset of diabetes. Levels of fasting serum C-peptide were higher in the patients with interferon-induced type 1 diabetes than those with type 1A diabetes at the onset (0.42 ± 0.27 vs. 0.25 ± 0.24 nmol/l, mean ± SD, p=0.016), at 1year (0.49 ± 0.24 vs. 0.23 ± 0.23 nmol/l, p=0.0015) and at 2-4 years (0.37 ± 0.32 vs. 0.13 ± 0.15 nmol/l, p=0.018) after the onset of diabetes. Insulin dose was not different at the onset (0.37 ± 0.21 vs. 0.47 ± 0.20 U/kg/day, p=0.12), but lower at 1year (0.30 ± 0.19 vs. 0.59 ± 0.14 U/kg/day, p=0.0007) and at 2-4 years (0.41 ± 0.25 vs. 0.59 ± 0.21 U/kg/day, p=0.029) after the onset of diabetes in the patients with interferon-induced type 1 diabetes than those with type 1A diabetes, while mean HbA_{1c} levels during first 5 years were not different between 2 groups (7.07 ± 0.97 vs. 7.48 ± 1.38%, p=0.51). Allele frequency of HLA-A*2402 was increased in patients with interferon-induced type 1 diabetes compared with those who did not develop diabetes despite interferon therapy [50.0% (12/24) vs. 20.0 % (4/20), odds ratio (OR) (95% confidence interval (CI)): 4.00 (1.09-17.26)]. Although frequency of other HLA-A alleles and DR-DQ haplotypes did not differ between these two groups, phenotypic frequency of DRB1*1302-DQA1*0102-DQB1*0604 was increased in these two groups combined [59.9% (13/22)] compared with normal controls [16.2% (22/136), OR (95%CI): 7.48 (2.89-20.27)], and those with type 1A diabetes [9.7% (10/103), OR (95%CI): 13.4 (4.72-40.96)].
Conclusion: Despite acute-onset form, interferon-induced type 1 diabetes was characterized by prolonged high titers of GAD antibodies, relatively preserved residual beta cell function, and subsequently lesser dose of insulin required. Addition of HLA-A*2402 to specific HLA class II background (DRB1*1302-DQA1*0102-DQB1*0604) may confer susceptibility to type 1 diabetes induced by interferon therapy.

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The frequency of ZnT8 autoantibodies and their HLA associations differ between Swedish and immigrant children with newly diagnosed type 1 diabetes in the Better Diabetes Diagnosis Study
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Background and aims: The ZnT8 autoantibodies (ZnT8A) are assuming an increasing importance in the prediction and diagnosis of childhood type 1 di-

abetes (T1D). The fact that single amino acid polymorphism at position 325 of ZnT8 identifies three antigenic variants: Argenine (ZnT8-R), Tryptophan (ZnT8-W) or Glutamine (ZnT8-Q); highlights the importance of ZnT8A in T1D. The type and frequency of autoantibodies against ZnT8 variants and their associations with the HLA class II DQ genes among different ethnic entities were not previously investigated. Our aim was to determine the relation between ethnic origin of the patient and the detection of ZnT8A in relation to high-risk HLA DQ genotypes and conventional islet autoantibodies (GAD65A, IA-2A and IAA) in Sweden.
Materials and methods: A total of 1868 (53% males) newly diagnosed T1D children <18 years at onset were recruited from the Better Diabetes Diagnosis (BDD) study during the period from May 2005 to September 2008. This cohort was grouped into three subgroups based on the origin of patients defined by country of birth of their parents and grandparents into Swedes (67%), non-Swedes (8%) and mixed-origin (16%) with 9% were of uncertain origins. Chi square test of independence was used to detect significant differences among ethnic groups in relation to ZnT8A variants and HLA and other islet autoantibodies. Logistic regression models were used to assess a possible association between nine high-risk HLA DQ genotypes and ZnT8A among ethnic groups.
Results: A total of 964 (52%) tested positive for ZnT8RA, 895 (48%) for ZnT8WA and only 609 (33%) for ZnT8QA. Among the same cohort, 1338 (72%) patients were positive for IA-2A, 1052 (56%) for GAD65A and 587 (31%) for IAA. In total only 126 (7%) patients were negative for all six autoantibodies. Nine HLA DQ genotypes were identified as high risk genotypes among the BDD patients compared to the Swedish general population. Only the ZnT8WA variant was significantly higher among Swedes (49%) compared to non-Swedes (39%) ($p<0.02$). Among Swedes ZnT8WA was associated with DQ8/8 ($p<0.009$) and DQ8/6.4 ($p<0.001$) genotypes while ZnT8RA and ZnT8QA were associated only with DQ8/6.4 genotype ($p<0.009$ and $p<0.007$ respectively). However, the DQ2 haplotype but not the DQ2/2 genotype was negatively associated with ZnT8RA and ZnT8QA variants among Swedes ($p<0.008$ and $p<0.005$ respectively) but not the ZnT8WA variant. Among non-Swedes none of these genotypes showed an association with any of the three ZnT8A variants. On the other hand, among Swedes both IA-2A and GAD65A were associated with all the three variants of ZnT8A. However, among non-Swedes only IA-2A showed associations with ZnT8RA and ZnT-8WA but not ZnT8QA (p values are not shown).
Conclusion: The DQ8/6.4 genotype and specifically the DQ6.4 haplotype are associated with ZnT8A among Swedish patients with T1D. Immigrant patients; however, develop ZnT8A in association with IA-2A but they may have different genetic associations from Swedish patients.
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Novel type 1 and type 2 diabetes susceptibility genes influence development of islet autoimmunity and type 1 diabetes in children of parents with type 1 diabetes
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Background and aims: Type 1 Diabetes (T1D) is an autoimmune disease with multiple susceptibility genes. It has been suggested that T1D and type 2 diabetes (T2D) may share some pathophysiological and genetic aetiology. The aim was to investigate whether novel T1D and T2D suceptibility genes influence development of islet autoimmunity and/or progression from islet autoimmunity to T1D.
Materials and methods: The single nucleotide polymorphisms (SNPs) in 6 T1D associated gene regions (*PTPN22, ERBB3, PTPN2, KIAA0350, CD25, IFIH1*) and 9 T2D associated gene regions (*KCNJ11, PPARG, TCF7L2, SLC30A8, CDKAL1, CDKN2A/2B, HHEX-IDE, IGF2BP2, FTO*) were analyzed in 1350 children from the German BABYDIAB study, a prospective observational study that follows offspring of mothers or fathers with T1D from birth to adulthood. A total of 137 children developed at least one persistent islet autoantibody [median age at first islet autoantibody appearance 4.9 (IQR 2.0-8.1)]; and 43 of these children progressed to T1D [median age at diagnosis 11.9 (IQR 9.4-14.9)].
Results: The T2D protective *TCF7L2* TT genotype was associated with a significantly higher incidence of islet autoimmunity than the CT and CC genotype (12% vs. 7% by age of 10 years, p=0.012). After stratification of the HLA genotype the *TCF7L2* TT genotype was associated with a significantly higher incidence of islet autoimmunity than the CT and CC genotype in children carrying the moderate/neutrale/protective HLA genotypes (10% vs. 5% by age 10 years p=0.006) but not in children carrying the high risk HLA genotypes (DR3/4 or DR4/4) (p=0.483). None of the other T1D and T2D susceptibility genes were associated with the development of islet autoimmunity in our cohort. Amongst the islet autoantibody positive children, progression to T1D was increased in children with the T1D susceptible *IFIH1* GG genotype (47% within 10 years after seroconversion vs. 32% in children with the GA and AA genotypes; OR: 1.9, p=0.01), children with the T1D susceptible *PTPN22* AA genotype (OR: 15.9, 95%CI: 1.6-160.4; p=0.001 vs CA and CC genotypes), children with the T2D susceptible *SLC30A8* CC genotype (OR: 2.4, 95%CI: 1.2-4.8; p=0.015 vs CT and TT genotypes), and children with the T2D susceptible *FTO* AA genotype (OR: 2.4, 95%CI: 1.1-5.4; p=0.031 vs CA and CC genotypes).
Conclusion: These data suggest that T2D susceptibility genotypes are unlikely to predispose to the development of islet autoimmunity, but that both T1D and T2D susceptibility genes may increase the rate of progression from islet autoantibody positive to diabetes.
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Genetic predisposition score for obesity is associated with BMI in individuals with type 1 diabetes
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Background and aims: With the help of large genome-wide association studies (GWAS) several obesity-predisposing variants have been identified. Attempts to replicate and further evaluate the association of these variants with different measures of obesity have also been carried out. The replications have been performed in different populations using both individual SNPs and genetic scores of identified SNPs. Whether the same obesity-predisposing SNPs that affect obesity in the general population affect obesity in individuals with type 1 diabetes (T1D) is not known. Therefore our aim was to study the association of obesity-predisposing SNPs with obesity in T1D using both individual SNPs and a genetic predisposition score based on the identified SNPs.
Materials and methods: All patients were part of a nationwide study of adult patients with T1D. Body mass index (BMI) and genotype data were available for 3232 individuals. 12 single nucleotide polymorphisms (SNP) in or near the following genes: *FTO*, *MC4R*, *SH2B1*, *MTCH2*, *KCTD15*, *NEGR1*, *TMEM18*, *GNPDA2*, *BDNF*, *FAIM2*, *SEC16B*, *ETV5* were used to create a genetic predisposition score for BMI. The SNPs had previously been associated with BMI in two large GWAS with BMI. The genetic predisposition score was created by calculating the number of risk alleles of the 12 SNPs for each patient. The genetic predisposition score for BMI among the patients ranged from 6 to 20. BMI was divided into three obesity categories: normal weight (BMI < 25, N=1609), overweight (BMI 25-29, N=1245) and obesity (BMI > 29, N=336).
Results: In the single SNP analyses only the SNPs located in FTO and near TMEM18 were significantly associated with BMI (P=0.0008 for FTO and P=0.01 for TMEM18) in directions consistent with prior reports. The significance persisted after adjustment for age, HbA1c and eGFR, factors known to affect BMI in T1D. While the *FTO* SNP was significant in both males (P=0.02) and females (P=0.02), the SNP near *TMEM18* was significant in males (P=0.02) but not in females (P=0.3). Although none of the other SNPs were associated with BMI, the genetic predisposition score differed significantly between the three obesity categories. Obese individuals had a genetic predisposition score of 13.1±2.0 (mean ± SD) compared to 12.8±2.0 for overweight and 12.7±1.9 for normal weight individuals (P=0.006). The score was independently associated with BMI when adjusted for factors affecting BMI in T1D (OR=1.11 [95%CI 1.04-1.19], P=0.002).
Conclusion: The obesity-predisposing SNPs in FTO and near TMEM18, originally identified in the general population, are also associated with BMI in individuals with T1D. Similarly, a genetic predisposition score of 12 obesity-predisposing SNPs was associated with BMI and obesity in T1D.

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Association of type 2 diabetes genes WFS and HHEX-IDE with disease progression in children with new onset type 1 diabetes

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Background and aims: Recently, several T2D related SNPs were investigated in a large T1D case-control study and PPARG and HHEX-IDE showed association with T1D. Previously we reported association of PPARG with residual beta-cell function and glycemic control during disease progression in T1D patients. The objective of this study was to investigate the impact of 11 newly identified T2D-related SNPs on disease progression in children with new onset T1D.
Materials and methods: The study is a multicenter longitudinal investigation with 18 participating paediatric centres from 15 countries in Europe (84% Caucasians). Clinical information and blood samples were collected from 275 children at diagnosis and at 1, 6, 12 months after onset. Genotyping of common SNPs in CDKAL1, WFS1, SLC30A8, CDKN2A-2B, IGFBP2, TCF7L2, FTO, HHEX-IDE, THADA was done by KBioscience using an in-house KASPar assay system. Statistics: C-peptide, HbA_{1c}, $IDAA_{1c}$ and proinsulin were analysed by multiple regression using age at onset, gender, DKA at onset, HLA class II risk groups, and genotypes as explanatory factors in a compound symmetric repeated measurement model.
Results: In a dominant model the G allele carriers (the wildtype allele) of the rs10010131 variant of the wolframin gene, WFS1, was significantly positively associated with stimulated C-peptide (est.: 1.73, p<0.0001), negatively with HbA_{1c} (est.: -0.49, p=0.005), negatively with $IDAA_{1c}$ (est.: -0.67, p=0.017) and positively with proinsulin (est.: 1.55, p=0.0045) the first 12 month after disease onset compared to the AA genotype carriers. In a co-dominant model the G allele carriers of the rs1111875 variant of the HHEX-IDE locus was significantly associated with stimulated C-peptide 12 months after disease onset compared to the AA genotype carriers in a allele dose-dependent manner (est.: 1.76 (GG), 1.30 (AG); p=0.0031).
Conclusion: The wildtype allele of the rs10010131 variant of the WFS1 gene is highly associated with a better residual beta-cell function and a corresponding better metabolic control during disease progression in new onset T1D compared to AA carriers. In addition the rs1111875 variant of the HHEX-IDE locus is significantly associated with a better residual beta-function with an allele-dose effect the first 12 month after diagnosis. This longitudinal study shows that genetic variants related to T2D might have an impact on disease progression in new onset T1D patients even if these same variants are found not to be predisposing to the onset of T1D. Thus, there might be some mechanistic overlap between T1D and T2D, which potentially can have therapeutic benefits for children with new onset T1D.

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The PTPN22 1858T allele enhances the emergence of clinical type 1 diabetes after the initiation of beta cell autoimmunity

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Background and aims: Data on genetic factors enhancing beta-cell destruction and emergence of type 1 diabetes (T1D) after the initiation of autoimmunity is limited. We set out to analyze the role of two polymorphisms associated with T1D, PTPN22 1858C/T and insulin gene (INS) -23HphI A/T in progression to T1D after the appearance of beta-cell autoimmunity.
Materials and methods: The study population comprised 285 children from the Finnish DIPP cohort with HLA-confirmed T1D risk. All subjects had developed positivity for at least one of the T1D-associated biochemically defined autoantibodies and 136 subjects presented with T1D. Two hundred-eight subjects developed at least two biochemically defined autoantibodies, and among these 124 presented with T1D.
Results: After the appearance of the first biochemically defined beta-cell autoantibody the PTPN22 1858T allele was strongly associated with progression to T1D. Fifty-eight (60%) subjects with the TT or CT genotype presented with T1D compared to 71 (40%) subjects with the CC genotype (P<0.001, HR 2.044, 95% CI 1.425-2.931, Cox regression analysis, multivariate test for the effect of PTPN22, INS and HLA DR3/DR4 genotypes). The effect remained significant also when analyzed after the appearance of the second biochemically defined autoantibody (P=0.001). INS -23HphI AA genotype was similarly associated with progression to clinical disease after the appearance of the first autoantibody (P=0.03), but the association disappeared when analyzed after the emergence of the second autoantibody (P=0.14).
Conclusion: The PTPN22 1858T allele is strongly associated with beta-cell destruction and progression to clinical disease after the initiation of beta-cell autoimmunity. The effect of the INS genotype predisposing to beta-cell autoimmunity remained weaker and disappeared after autoantibody spreading.

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Mutation in SIRT1 in familial type 1 diabetes

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Background and aims: Type 1 diabetes was diagnosed in a 26-year-old Ashkenazi Jewish male on the basis of hyperglycaemia, a lean body mass index of 21.5 Kg/m^2 and β-cell auto-antibodies. The patients' sister, father, and a paternal cousin were also diagnosed with type 1 diabetes at the ages of 7, 12, and 15 years, respectively. The aims were to further characterise the patient, to identify the mutation and its pattern of inheritance and to characterise the molecular phenotype of the mutated protein.
Materials and methods: To characterise the index patient an OGTT, a euglycemic-hyperinsulinemic clamp and a muscle biopsy were performed. The mutation was identified with a candidate gene approach and exonic sequencing and introduced by site directed mutagenesis into a retroviral vector (MSCV) containing the wild type gene. Mutated and wt protein were stably expressed in 293T cells and insulin producing MIN6 cells.
Results: The index patient presented with signs of beta-cell autoimmunity (auto-antibodies to glutamic acid decarboxylase 1150 U/L and islet-cell autoantibody-2 3.0 U/L), insulin dependence and impaired beta-cell function. Further, there was an unexpected insulin resistance as revealed by a euglycemic-hyperinsulinemic clamp study (M value 34.1 10^{-3} mM / min / Kg BW) and a muscle biopsy. The affected family members lacked measurable C-peptide and were also islet auto-antibody positive. The pattern of inheritance was indicative of an autosomal dominant mutation. Analysis of SIRT1, a protein deacetylase implicated in ageing, beta-cell function and insulin resistance, revealed the presence of a T to C exchange in exon 1 of a single allele, corresponding to a Leucine-Proline mutation at residue 107 in the protein. A common polymorphism of the T to C substitution, mutations in the MODY genes and HLA associations were ruled out. Analysis of the structure of the SIRT1 protein revealed that L107P lies outside of the conserved Sirtuin enzymatic core, in a densely-charged region of the protein involved in protein-protein interactions. Isolated SIRT1-L107P protein showed only a slight decrease in deacetylase activity relative to the wild-type protein, and there were no changes in protein stability and the subcellular localization of SIRT1. Stable overexpression of L107P sirt1 protein in the beta-cell line MIN6 did not change insulin mRNA expression and insulin secretion relative to an equal expression of the wild-type protein. NF-kappaB is a known deacetylation target of SIRT1 and SIRT1 L107P MIN6 cells displayed elevated expression of NF-kappaB regulated genes iNOS, chemokine KC and cytokine TNF-alpha together with overproduction of nitric oxide.
Conclusion: Here we describe the first human mutation in the SIRT1 gene in a family with clusters of type1 diabetes. The study implicates that SIRT1 plays a role in auto-immunity and glucose homeostasis in humans and it may serve as a paradigm to initiate further screening of SIRT1 mutations in families with clusters of type 1 diabetes.
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Genetic regulation of 25(OH)D_3 and 1,25$(OH)_2D_3$ serum concentrations in type 1 diabetes patients

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Background and aims: Growing evidence suggests an important role for the vitamin D system in the pathogenesis of type 1 diabetes. In humans, low serum levels of 25(OH)D_3 and its active form 1, 25$(OH)_2D_3$ correlate with a higher risk for this disease. In order to become biologically active, vitamin D_3 needs to be hydroxylated first into 25(OH)D_3 in the liver by the enzyme CYP2R1. After this hydroxylation, 25(OH)D_3 binds to the vitamin D binding protein (DBP) and circulates. In the kidney 25(OH)D_3 is hydroxylated to 1,25$(OH)_2D_3$ by CYP27B1. Then 1,25$(OH)_2D_3$ binds to the vitamin D receptor (VDR) to exert its immunmodulator effects via vitamin D response elements (VDRRE). Catabolism of both the 25(OH)D_3 and 1,25$(OH)_2D_3$ occur via the 24-hydoxylase (CYP24). Therefore we investigated whether genetic variation in vitamin D synthesis, metabolism, transport and catabolism influence 25(OH)D_3 and 1,25$(OH)_2D_3$ serum levels in type 1 diabetes patients.
Material and methods: 223 type 1 diabetes patients were genotyped for polymorphisms (n= 13) within the VDR- (rs7975232, rs731236, rs1544410, rs10735810), the CYP2R1- (rs10741657, rs12794714), the DBP-(rs4588, rs7041), the CYP27B1- (rs10877012) and the CYP24- (rs229641, rs2248137, rs2585426, rs927650) genes by using restriction fragment length polymorphism or real time PCR. The 25(OH)D_3 and 1,25$(OH)_2D_3$ serum levels were measured by radioimmunoassay (DIASORIN). Concentrations of 25$(OH)_2D_3$ < 20 ng/ml were defined as vitamin D insufficiency, while a range of 19.9-67 pmol/ml of 1,25$(OH)_2D_3$ was considered normal. The non-parametric Wilcoxon-Mann-Whitney -test was used for the statistic analysis. A p value < 0.05 was considered as significant.
Results: Deficiency of 25(OH)D_3 or 1,25$(OH)_2D_3$ was found in 54% and 9.4% respectively. Of the 13 analyzed polymorphisms the rs10877012, rs10741657 and the rs7041 within the CYP27B1, CYP2R1 and the DBP genes, respectively, had a statistically significant influence on the serum concentrations of 25(OH)D_3 or 1,25$(OH)_2D_3$. Thus patients carrying the allele C of the CYP27B1, which has been reported to be a risk factor to develop type 1 diabetes, had lower 1,25$(OH)_2D_3$ serum levels (34 pmol/L) than those patients carrying the alleles A (42 pmol/L) or AC (38 pmol/L; p<0.05). Also patients carrying the allele T of the rs7041 within the DBP gene showed lower levels of 25(OH)D_3 (p<0.02), whereas patients with the genotype A of the rs10741657 within the CYP2R1 had higher levels of 25(OH)D_3 in comparison to those patients without these genotypes (p<0.03).
Conclusion: This study demonstrates that vitamin D serum levels, both 25(OH)D_3 and 1,25$(OH)_2D_3$, are regulated by genetic variations at least within the genes coding for CYP27B1, CYP2R1 and DBP altering 25(OH)D_3 and 1,25$(OH)_2D_3$ concentrations. Therefore these genetic variants are functionally relevant and may thereby predispose to impaired function of the immune system in type 1 diabetes through systemic and/or tissue specific differences in vitamin D action.
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PS 3 Genome-wide association studies and their follow-up

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Meta-analysis of sex-specific genome-wide association studies of type 2 diabetes

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Background and aims: Genome-wide association studies (GWAS) of type 2 diabetes (T2D) have identified more than thirty confirmed loci contributing effects to the disease. However, despite this success, much of the genetic component of T2D susceptibility remains unexplained. One potential source of genetic variation contributing to this "missing heritability" is that with effects that differ in magnitude and/or direction in males and females. Sex-specific effects have been observed in a variety of traits, including lipid levels and schizophrenia, but may not be readily identified through traditional analysis of GWAS of males and females, combined, because of a lack of statistical power. We have therefore undertaken the first large-scale meta-analysis of sex-specific T2D GWAS to address the following aims: (i) assess the heterogeneity of effects between the sexes at confirmed disease loci; and (ii) identify novel male- and female-specific associations with the disease for follow-up.
Materials and methods: We performed GWAS of T2D in six cohorts of northern European ancestry (total effective sample size of 9332 males and 7744 females). Genotype data were imputed in each cohort for up to 2.5 million SNPs, genome-wide, including the X chromosome. SNPs were subsequently tested, in males and females separately, for association with disease under an allelic-dose model, adjusting for cohort specific covariates. For each sex, allelic odds ratios (OR) were then combined across cohorts through fixed-effects inverse-variance weighted meta-analysis. Heterogeneity of combined allelic OR between males and females was assessed by means of Cochran's *Q*-statistic.
Results: Among confirmed T2D loci, allelic effects between males and females were generally homogeneous. However, there was nominal evidence of heterogeneity at two loci, both demonstrating stronger effects on T2D in males: *BCL11A* (p = 0.023, male OR = 1.16 [1.10-1.24], female OR = 1.05 [0.99-1.12]); and *KCNQ1* (p = 0.036, male OR = 1.15 [1.08-1.22], female OR = 1.04 [0.98-1.12]). The strongest novel signals of association with T2D in sex-specific meta-analyses were observed in males, with SNPs at two loci achieving genome-wide significance ($p<5\times10^{-8}$): proximal to *SLC35D3* ($p=1.7\times10^{-8}$, OR = 1.19 [1.12-1.26]); and proximal to *DGKB* ($p=2.5\times10^{-8}$, OR = 1.26 [1.16-1.37]). The male-specific signal at *DGKB* is independent of the overall T2D association previously reported at this locus (r^2=0.023). Novel signals with suggestive evidence of association ($p<10^{-5}$) were observed at 7 additional loci in males, and at 14 loci in females, both sets incorporating genes with plausible biological candidacy for T2D.
Conclusion: Sex-specific GWAS of T2D did not highlight strong evidence of heterogeneity of allelic effects between males and females at already confirmed loci. SNPs from 23 regions with at least suggestive evidence of association, identified using male- and female-specific meta-analyses, are currently being followed up in additional cohorts through in silico replication and de novo genotyping.

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Age-dependent genetic effects on post-load glucose during 18 years of follow-up of the Whitehall II Cohort

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Background and aims: Cross-sectional, genome-wide association studies have recently identified common, single nucleotide polymorphisms (SNPs)

associated with fasting and/or 2-hour post-load glucose levels in non-diabetic individuals. It is unknown whether effects of these variants are constant over time or change with advancing age, as most genetic studies rely on glucose measurements at a single time point.
Material and methods: A total of 4,519 non-diabetic British civil servants (aged 40-78 years) participating in the Whitehall II study and attending up to four 5-yearly clinic visits including repeated oral glucose tolerance tests were studied. A weighted genetic score of glucose raising alleles was calculated separately for fasting and 2-hour glucose levels, including 16 and 5 SNPs respectively (mean score (SD) 17.0 (3.0) for fasting and 4.0 (1.4) for 2-hour glucose). Multilevel models accounting for the dependence of measurements within individuals and adjusted for sex and BMI were used to study the main effects of each genotype score on fasting and 2-hour glucose levels and their interactions with age.
Results: Over a mean follow-up of 10 years (range 0-18 years), 2-hour but not fasting glucose levels showed a significant increase with age (0.048 (95%CI: 0.037-0.058) mmol/l per year in participants without any risk alleles). The fasting glucose score significantly predicted glucose levels at 55 years (0.030 (95%CI: 0.026-0.034) mmol/l difference per genetic score point), an effect that remained constant over time (figure 1, left). At age 55 years, 2-hour glucose levels differed by 0.070 (95%CI: 0.044-0.096) mmol/l per genetic score point; notably, this effect became stronger with increasing age (0.004 (95%CI: 0.001-0.006) mmol/l per genetic score point per year), resulting in diverging age trajectories by genetic score (figure 1, right).
Conclusion: Genetic effects on 2-hour glucose appear to depend on age, such that the difference in 2-hour glucose levels per additional risk allele increases with advancing age. Our findings suggest that the effects of age or related environmental factors need to be taken into account when studying genetic influences on 2-hour glucose levels.

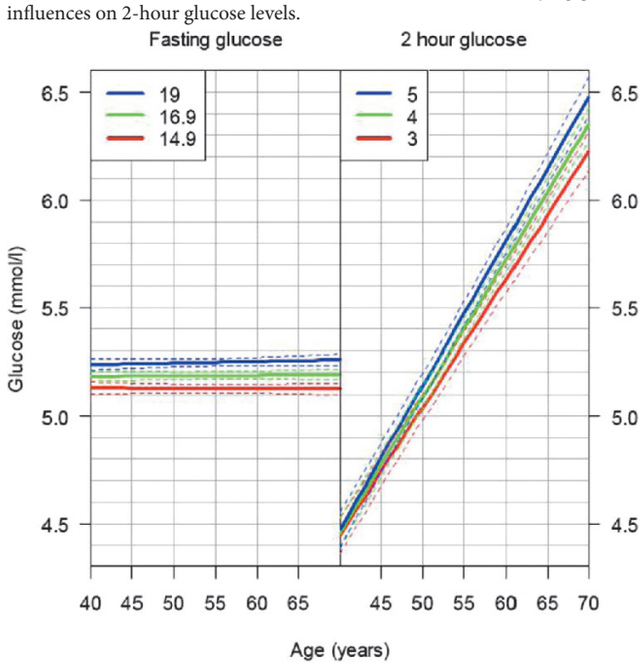

Figure 1: Estimated glucose levels and confidence intervals in males with BMI of 23 kg/m², for quartiles of the gene score distribution. Left: Fasting glucose. Right: 2-hour glucose. Colours indicate quartile values of the gene score.

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Genetic predisposition to long-term deterioration in glucose homeostasis: 10-yr follow-up of the GLACIER Study

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Background: Major advances have recently been made in discovering genetic loci for hyperglycemia. These findings emerged primarily through genome-wide scans of cross-sectional data. Little is known of how these loci affect changes in glycaemia over time.
Methods: Sixteen recently discovered fasting glucose-raising loci were genotyped in middle-aged non-diabetic participants in the GLACIER Study, a population-based prospective cohort from Northern Sweden. Genotypes were tested for association with baseline fasting and 2-hr glucose concentrations (N=16,048), and with change in glucose concentrations and the development of impaired fasting glucose (IFG) over 10-yrs follow-up (N=4,109).
Results: Cross-sectional directionally consistent replication with fasting glucose levels was achieved for 12/16 variants. After adjusting for fasting glucose levels, the fasting glucose risk alleles for 4 loci were positively and 3 loci were negatively associated with 2-hr glucose concentrations. In a genetic risk score (GRS) derived by adding unweighted risk alleles, those in the 80th GRS percentile had fasting glucose levels 0.17 mmol/l higher than those in the 20th GRS percentile (P=5.3x10^{-35}). In prospective analyses (Table 1), fasting glucose-raising alleles at 5 loci were nominally associated with worsening fasting glycaemia, 3 also predicted the development of IFG. After adjustment for baseline and follow-up fasting glucose, 2 variants were nominally associated with change in 2-hr glucose levels, (*ADCY5* rs11708067, *MTNRB1* rs10830963). Surprisingly the *MTNRB1* variant, which was predictive of worsening fasting glycaemia, was protective of deterioration in 2-hr glycaemia. The GRS (80th vs. 20th percentiles) was associated with 0.13 mmol/l (P=4.3x10^{-5}) greater elevations in fasting glucose and 1.34-fold (95% CI: 1.07-1.70) greater risk of developing IFG during 10-yrs follow-up. Adjusting the fasting glucose models for 2-hr glucose, or vice versa, or weighting the GRS by previously published effect estimates did not materially affect these results.
Conclusion: Multiple genetic loci predict deteriorations in fasting glucose concentrations during 10-yrs follow-up of a Northern Swedish cohort. Studies testing prospective relationships with the development of diabetes complications will be required to determine the clinical value of these genetic prediction models.

Table 1 The ability of each single nucleotide polymorphism (SNP), singularly and in combination, to predict changes in fasting and 2-hr glucose levels and development of impaired fasting glucose (IFG) over a 10-yr follow-up period (n=4,109).

SNP	Nearest gene(s)	Effect allele (other)	Freq	Fasting glucose (mmol/l)		2 hr glucose (mmol/l)		IFG	
				Beta (SE)	*P*-value	Beta (SE)	*P*-value	OR	95% CI
rs4607517	*GCK*	A(G)	0.15	0.062 (0.020)	0.002	0.016 (0.048)	0.731	1.11	(0.97-1.28)
rs10830963	*MTNR1B*	G(C)	0.28	0.044 (0.017)	0.008	-0.092 (0.039)	0.018	1.22	(1.09-1.37)
rs2191349	*DGKB-TMEM195*	T(G)	0.51	0.032 (0.015)	0.028	-0.006 (0.034)	0.871	1.10	(0.99-1.21)
rs10885122	*ADRA2A*	G(T)	0.89	0.058 (0.023)	0.013	0.044 (0.055)	0.421	1.25	(1.05-1.48)
rs560887	*G6PC2*	C(T)	0.71	0.035 (0.016)	0.030	0.005 (0.038)	0.899	1.18	(1.05-1.32)
rs340874	*PROX1*	C(T)	0.53	0.023 (0.014)	0.107	-0.037 (0.034)	0.273	1.06	(0.96-1.18)
rs11071657	*C2CD4B*	A(G)	0.60	0.023 (0.015)	0.129	-0.027 (0.036)	0.445	1.03	(0.93-1.15)
rs7903146	*TCF7L2*	T(C)	0.20	0.025 (0.018)	0.170	0.045 (0.043)	0.305	1.19	(1.05-1.35)
rs780094	*GCKR*	C(T)	0.71	-0.020 (0.016)	0.207	-0.065 (0.038)	0.085	0.96	(0.86-1.08)
rs13266634	*SLC30A8*	C(T)	0.70	0.019 (0.016)	0.227	0.042 (0.037)	0.265	1.07	(0.95-1.19)
rs7944584	*MADD*	A(T)	0.76	0.015 (0.018)	0.406	-0.070 (0.042)	0.093	0.97	(0.86-1.10)
rs174550	*FADS1*	T(C)	0.66	-0.010 (0.016)	0.525	-0.005 (0.037)	0.898	0.94	(0.84-1.05)
rs11920090	*SLC2A2*	T(A)	0.86	0.011 (0.021)	0.593	0.006 (0.051)	0.902	1.06	(0.91-1.23)
rs11605924	*CRY2*	A(C)	0.50	0.007 (0.015)	0.623	-0.028 (0.034)	0.418	1.08	(0.98-1.20)
rs11708067	*ADCY5*	A(G)	0.79	0.009 (0.018)	0.626	0.098 (0.043)	0.022	0.96	(0.85-1.09)
rs7034200	*GLIS3*	A(C)	0.43	0.001 (0.015)	0.938	-0.001 (0.035)	0.969	1.00	(0.90-1.11)
GRS	-	-	-	0.017 (0.004)	4.27x10^{-5}	-0.005 (0.010)	0.643	1.05	(1.02-1.09)

SNPs are ranked by P-value of the per-allele effect (beta) on fasting glucose. Models are adjusted for age, sex and follow-up time. In the model where fasting glucose or 2-hr glucose is the outcome, models are also adjusted for baseline fasting or 2-hr glucose levels, respectively. All genotypes are located on the plus strand and are coded according to HAPMAP CEU (Phase II+III), release 27, NCBI build 36). SE = standard error; OR = odds ratio; CI = confience limit, GRS = genetic risk score

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Glycaemia determines the effect of type 2 diabetes risk genes on insulin secretion

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Background and aims: Several polymorphisms in genes associated with diabetes risk reduce glucose- and/or incretin-induced insulin secretion. In this study, we investigated whether there are interactions between glycemia and such diabetes-risk polymorphisms. We hypothesized that diabetes risk genes specifically associated with impaired incretin-induced insulin secretion would show a glycemia dependent effect on insulin secretion.
Materials and methods: Insulin secretion was assessed by insulinogenic index and AUCC-pep/AUCGlc in 1576 subjects with various glucose tolerance statuses using an oral glucose tolerance test. Participants were genotyped for SNPs which were previously shown to be associated with type 2 diabetes and impaired insulin secretion and specifically impaired incretin-induced insulin secretion (rs7903146 [TCF7L2], rs10010131 [WFS1]). Furthermore, the impact of the interaction between genetic variation in TCF7L2 and glycemia on changes in insulin secretion was tested in 315 individuals taking part in a lifestyle intervention study for 9 months.
Results: For two SNPs (TCF7L2, WFS1) we found a significant interaction with glucose control on insulin secretion (all p less of equal 0.0018 for glucose x genotype). When plotting insulin secretion against glucose at 120 minutes during the OGTT, the compromising effect of the risk alleles on insulin secretion is most apparent under high glucose conditions for both SNPs. In the longitudinal study, rs7903146 in TCF7L2 showed a significant interaction with baseline glucose tolerance on change in insulin secretion during lifestyle intervention (p=0.0008). Increased glucose levels at baseline predicted an increase in insulin secretion in carriers of the risk alleles, whereas the change in insulin secretion during lifestyle intervention was not influenced by blood glucose levels in carriers of the non-risk alleles. None such interaction was found for the WFS1 SNP.
Conclusion: For the diabetes risk genes TCF7L2 and WFS1 which are associated with impaired incretin signaling, glycemia determines SNP effects on insulin secretion. This implicates the relevance of these SNPs in different stages of the pathogenesis towards type 2 diabetes mellitus.

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The risk allele score for type 2 diabetes mellitus were associated with age of diagnosis and basal insulin secretion in Japanese population

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Background and aims: T2DM is a multifactorial disease whose onset depends not only on the genetic predisposition, but also environmental factors, and it remains unclear to what extent genetic predisposition affects the clinical presentation. Large-scale studies in Europe have reported that the odds ratio of each polymorphism for the onset of diabetes is low, ranging from 1.1 to 1.5. Consequently, it is considered that the onset of diabetes mellitus may be related to the accumulation of several polymorphisms. We counted the number of risk alleles of the SNPs in Japanese participants and defined the total number of risk alleles as a risk allele score. In this study, the effect of the risk allele score on the clinical presentation of T2DM was investigated.
Materials and methods: The subjects were 720 T2DM outpatients of our hospital and associated hospitals, and 760 non-diabetic controls. Ten SNPs out of 10 candidate T2DM-susceptibility genes, namely, HHEX , CDKAL1 ,CDKN2A/B, SLC30A8 KCNJ11 , IGF2BP2, PPARG, TCF7L2, FTO and KCNQ1 were genotyped by TaqMan PCR assay. Clinical information, including the current BMI, age at diagnosis of diabetes, age at the maximum body weight, family history, disease duration, FPG, HbA1c, F-CPR(= fasting serum CPR), CPI (=F-CPR*100/FPG), UCPR (=urinary C-peptide)/Cr and treatment was collected.
Results: Of the 10 genes, a significant difference between the T2DM group and control group was found for 5 genes, namely, CDKAL1, CDKN2A/B, SLC30A8, IGF2BP2 and KCNQ1. Based upon the frequency distribution of the risk alleles of the 5 SNPs, which were significantly associated with type 2 diabetes, we classified the participants into low-(a total of four or fewer risk alleles), intermediate-(a total of five or six risk alleles) and high-(a total of seven or more risk alleles)risk genetic groups. In the comparison of the clinical presentation between the low-risk group and high-risk group, although the maximum BMI of the latter group (27.0) was significantly lower than that of the former group (28.3) (p<0.01), the age at diagnosis of diabetes in the latter group (49.4 years) was significantly younger than that of the former group (52.1 years) (p<0.05). However, there was no significant correlation between the total number of risk alleles (0-2) for each gene and the age at diagnosis of diabetes mellitus. Regarding the indices of the basal linsulin secretary capacity, the F-CPR, CPI and UCPR/Cr were all significantly lower in the high-risk group than in the low-risk group, after adjustments for age, sex, FPG, BMI, and duration of diabetes (p<0.01). The ratio of insulin therapy in the high-risk group (37%) was greater than that in the low-risk group (25%) (p<0.05).
Conclusion: It was demonstrated that while the risk alleles for each individual polymorphism had little effect on the onset of diabetes, the high risk allele score of 5 SNPs, which were associated with significantly associated with type 2 diabetes in this study, was related with earlier age at onset of diabetes, decreased insulin secretion and increment of the ratio of insulin therapy.

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Replication of European GWAS-derived type 2 diabetes susceptibility SNPs in Pakistani populations

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Background and aims: Type 2 diabetes (T2D) is a major public health issue in the Indian subcontinent (India, Pakistan and Bangladesh), where it is predicted that the disease will affect approximately 76 million adults by 2025. A high prevalence of the disease is also observed in populations of South Asian ancestry living in other areas of the world. Although lifestyle factors, such as diet and exercise, undoubtedly contribute to the development of T2D, these factors cannot fully explain the high prevalence of the disease in South Asian populations. This excess risk has been partly attributed to the genetic background of the population. Recently the advent of genome-wide association studies (GWAS) has led to the discovery of a number of novel SNPs (single nucleotide polymorphisms) that confer risk of T2D development. The majority of these large scale studies, however, have investigated cohorts of white European origin, and South Asian populations have been considerably understudied. In this study we investigated 16 SNPs that have been robustly associated with T2D in Europeans to determine whether they have a similar effect on disease risk in two Pakistani populations of Punjabi ancestry, one UK-resident and one indigenous to Pakistan.
Materials and methods: We genotyped 2992 subjects (1609 with T2D and 1383 normoglycaemic controls) for 16 SNPs using either TaqMan (AppliedBiosystems, Warrington, UK) or KASPar (KBiosciences, Hoddesdon, UK) methods. One SNP was chosen from each of the following loci: *TCF7L2, CDKN2A/2B, CDKAL1, HHEX/IDE, IGF2BP2, CDC123/CAMK1D, SLC30A8, PPARG, KCNJ11, WFS1, TCF2, ADAMTS9, THADA, NOTCH2, TSPAN8/LGR5, JAZF1*. Logistic regression was used to investigate the association between each SNP and T2D, using an additive model and including sex, age and population as covariates. An allelic risk score variable was constructed using those SNPs that showed some evidence (p<0.1) for association with T2D, by combining the total number of risk alleles for each subject.
Results: Significant associations with T2D were observed for *TCF7L2* (OR=1.25 [1.12, 1.40] *p*=0.00008), *CDKN2A/2B* (OR=0.79 [0.67, 0.93] *p*=0.005), *HHEX/IDE* (OR=1.14 [1.03, 1.26] *p*=0.013), *IGF2BP2* (OR=1.18 [1.06, 1.31] *p*=0.002) and *TCF2* (OR=0.90 [0.80, 1.00] *p*=0.041). Trends towards association with T2D were also observed for *SLC30A8* (OR=0.89 [0.79, 1.01] *p*=0.064) and *PPARG* (OR=0.88 [0.75, 1.02] *p*=0.089). These variants appear to have an additive effect on T2D risk, the allelic risk score showing that each risk allele contributes a 1.15-fold increase in disease risk (OR=1.15 [1.10, 1.21] *p*=2.04 x 10-9).
Conclusion: We have demonstrated that a number of genetic variants that predispose to T2D in European populations have a similar effect in populations of Pakistani origin. Furthermore, to our knowledge this is the first time that a disease association with variants in *CDKN2A/2B*, *HHEX/IDE* and *TCF2* has been demonstrated in any South Asian population.
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Evaluation of the phenotypic effects of common FTO and MC4R genetic polymorphisms in a general population from South India

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Background and aims: Obesity is a multi-factorial trait that results from a complex interplay between genes and environment. Genome wide association (GWA) studies have shown that common variants in the FTO (Fat mass and Obesity associated) and MC4R (Melanocortin 4 receptor) genes are associated with increased risk of Obesity and type 2 diabetes in Caucasians. These associations, however, were not consistent and the effect was independent of body mass index (BMI) in the subjects with type 2 diabetes among Asians. In the present study, we aimed to evaluate the phenotypic effects of the common FTO and MC4R genetic polymorphisms in a young adult population from South India.
Materials and methods: We examined the common variants of the FTO (rs9939609) and MC4R (rs17782313) genes in two independent cohorts (n=3241) stratified based on the area of residence to rural (n=1221) semiurban (n=1023) and urban (n=997)from South India. Anthropometric measurements of adiposity including BMI, waist circumference (WC), waist-hip ratio (WHR), body fat % and skin fold thickness were performed. Serum glucose, lipids were measured in non fasting venous blood samples. The data were summarized by comparing means across groups by ANOVA and Kruskall Wallis tests respectively for normal distributed and skewed variables. Comparative analyses of genotype frequencies between study groups were performed using chi-square test. Phenotypic effects in each genotypes were deduced based on dominant/recessive models using multivariate logistic regression analyses.
Results: The rural group comprised of lean phenotypes with low mean BMI (19.8 ± 3.4) and WHR (0.83 ±0.07) while the urban population had a significantly higher mean BMI (21.8 ± 3.9, p<0.001), higher WC (77.1 ± 10.9, p<0.001) and higher fasting blood glucose (5.6 ± 1.0) compared to the rural and semi-urban groups. Despite a lean phenotype, the homozygous AA genotype of the FTO gene, was significantly associated with higher body fat % (23.2 ± 9.6, p=0.03), Skin fold measurements at triceps (median 9.55 interquartile range 6.53-14, p=0.004), biceps (median 4.53, range 3.13-7.22, p=0.03), subscapular (14.73, range 10.35-25.88, p=0.01) and abdomen (median 16.13, range 8.63-30.55, p=0.002) in the rural group. Significant association with anthropometric measurements was not seen among the urban and the semi urban group though they had a significantly higher BMI and subcutaneous fat. A similar trend was also observed amongst the rural population in the homozygous CC genotype of MC4R with modest significance.
Conclusion: Data from the present study suggests that the common variants of the FTO and MC4R genes have significant genetic influence on subcutaneous fat and body fat percentage, but not BMI in the South Indian population.
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Common variant of MTNR1B is associated with difficulties maintaining sleep but fails to influence the association between sleep disturbances and type 2 diabetes: the HUNT study

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Background and aims: Recent studies have demonstrated that genetic variation in the melatonin receptor 1B (MTNR1B) is associated with type 2 diabetes. Melatonin contributes to the regulation of sleep, and sleep problems are a documented risk factor for type 2 diabetes. Whether the MTNR1B gene variant, which confers risk of diabetes, induces sleep problems is not known, nor whether a putative effect on sleep impacts on the link between sleep problems and type 2 diabetes. We tested 1) whether the risk variant SNP rs10830963 in the MTNR1B gene is associated with self reported sleep problems, and 2) whether presence of the risk variant influences the association between sleep disturbances and type 2 diabetes.
Materials and methods: We used information from a case-control study nested within the population-based Nord-Trøndelag Health Study, including 1,074 cases of type 2 diabetes and 1,447 controls (matched by age and sex). Information on different aspects of sleep disturbances was obtained by questionnaire. Genotyping was performed using the Tacman discrimination assay (Applied biosystems). Odds ratios (OR) and 95% confidence intervals (CI), adjusted for age, sex and BMI, were calculated using logistic regression models.
Results: Reported difficulties maintaining sleep were more frequent in subjects with the G allele of the rs10830963 (OR 1.49, 95% CI 1.04-2.14). In confirmation of previous studies, this allele also conferred increased risk of type 2 diabetes (OR 1.19, 95% CI 1.01-1.41). However, the previously documented association between sleep problems and type 2 diabetes was not influenced by the MTNR1B G allele. Hence, the OR for the association between sleep disturbances and type 2 diabetes was 1.46 (95% CI 1.01-2.10) for subjects with the G allele of rs10830963, and 1.53 (95% CI 1.08-2.16) for subjects without the G allele.
Conclusion: The common variant rs10830963 in the MTNR1B gene is associated with difficulties maintaining sleep and also with type 2 diabetes. However, presence of the risk allele does not influence the association between sleep disturbances and type 2 diabetes.
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Expression of ARL15, a type 2 diabetes risk variant, is increased in cultured human skeletal muscle cells from insulin-resistant type 2 diabetes patients

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Background and aims: A recent genome wide association (GWAS) meta-analysis identified a new variant in the *ARL15* gene associated with decreased circulating adiponectin levels, and increased risk of coronary heart disease and type 2 diabetes. *ARL15* (ADP-ribosylation factor-like 15) encodes a GTP-binding protein of unknown function that is highly expressed in skeletal muscle. The aim of this study was to investigate *ARL15* expression in cultured human skeletal muscle cells, and to determine (1) whether expression changed with differentiation from myoblasts to myotubes, and (2) whether expression was altered in cultured muscle cells from insulin resistant type 2 diabetic patients.
Materials and methods: Cultured skeletal muscle cells derived from type 2 diabetic patients with a family history of diabetes and clinical evidence of insulin resistance and healthy non-diabetic control subjects with no family history of diabetes were studied. We have previously shown that defects of insulin action are retained in the cultured myotubes from the diabetic patients. *ARL15* expression was measured in cultured myoblasts and day 7 differentiated myotubes. Quantitative real-time PCR (QPCR) was used to measure gene expression relative to GAPDH as the reference gene, while protein expression was determined by Western blotting. Statistical analyses were performed using the Wilcoxon signed rank and Mann Whitney U tests.
Results: In cultures from healthy control subjects, *ARL15* was expressed in both myoblasts and myotubes and increased with differentiation. After normalisation to GAPDH, expression increased from 0.24±0.06 (mean±SEM) units in myoblasts to 0.68±0.25 in myotubes (n=9; p=0.03). Western blotting confirmed ARL15 protein expression in both myoblasts and myotubes, and was 1.6-fold higher following differentiation to myotubes (n=3; p=0.04). *ARL15* expression was compared between cultured muscle cells obtained from 6 insulin-resistant type 2 diabetic patients and 6 age- and BMI-matched controls. *ARL15* expression tended to be higher in diabetic vs control myoblasts (0.44±0.24 vs 0.12±0.04 units; p=0.06). *ARL15* expression increased with differentiation in both groups, and was significantly higher in the diabetic vs control myotube cultures (0.94±0.27 vs 0.25±0.05 units, p=0.004).

Conclusion: These data show that *ARL15* and the encoded protein are well expressed in cultured human skeletal muscle cells, and expression increases with differentiation. *ARL15* expression was increased in differentiated myotubes from insulin resistant type 2 diabetic patients. Further work is needed to explore whether the increased *ARL15* expression in the diabetic myotubes is directly related to the previously observed impairment of insulin action in these muscle cell cultures.
Supported by: Diabetes UK

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Whole genome sequencing identifies naturally-occurring polymorphisms in a polygenic model of spontaneous type 2 diabetes
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Background and aims: The GK rat is a well-established inbred model of type 2 diabetes (T2D), spontaneously exhibiting the main features of T2D, as the consequence of naturally-occurring diabetes-causative DNA variants isolated from an outbred Wistar stock By making use of massively parallel next-generation sequencing technologies, our aim was to sequence the entire genome of the GK rat at sufficient coverage for accurate identification of nucleotide and possibly structural variants. More particularly we will examine GK genomic regions linked to phenotypes related to T2D and other metabolic traits as well as genes found to be differentially expressed between GK and control strains through genome-wide gene expression (eQTL) studies.
Materials and methods: Genomic DNA is extracted from GK rat liver using Qiagen DNeasy blood and tissue kit. The DNA is fragmented, and adaptors are ligated to the fragments to make a library for 51-bp paired-end sequencing on an Illumina Solexa Genome Analyzer II next-generation sequencer. Solexa software is used for base calling (Bustard) and initial alignment (GERALD) with the ELAND algorithm. Further alignments are carried out using STAMPY, and SNP detection by an in-house algorithm at the Wellcome Trust Centre for Human Genetics.
Results: In total, 29 lanes of sequencing were run, giving a total of about 330 million 51-bp reads, and resulting in approximately 9x average coverage across the genome. Currently 80% of the sequence maps back to the BN rat RGSC 3.4 assembly. Looking specifically in a 6.32Mb region carried by a congenic rat strain bred to capture a GK diabetes QTL, we find 14,453 candidate SNPs, some in genes that have emerged from monogenic family studies and genome wide association studies in humans, including Glis3, as well as in Dmrt3, a novel candidate which is an expression QTL in GK. Gene expression and physiological QTLs are now annotated with a more complete set of SNPs and other variants.
Conclusion: We have generated whole genome sequence for the GK rat, aligned it to the BN reference sequence, and have identified sequence variants in candidate genes for T2D. Integration of whole genome polymorphism data with physiological and genome-wide gene expression quantitative trait loci in F2 crosses and congenic strains, provides a powerful novel resource for understanding the full genomic landscape of spontaneous polygenic T2D and for identifying new candidate genes and pathways contributing to T2D and the metabolic syndrome.
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PS 4 Genes and islets

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What can a meal test tell us about the heritability of the beta cell function?
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Background and aims: Type 2 diabetes is a multi-factorial disease in which deterioration of the beta cell function plays a crucial part. This same-sex twin family study is the first to assess the heritability of beta cell function parameters derived from the most physiological challenge test, the mixed-meal tolerance test.
Materials and methods: We recruited 77 same-sex twin families from the Netherlands Twin Register, including 51 MZ twin pairs, 21 DZ twin pairs, 5 twins without a co-twin and 34 same-sex siblings of the twins. All 183 healthy participants (77 male) were of European origin and aged 20-49 years. After anthropometric measurements were performed, a standardized mixed-meal was given and blood was sampled repeatedly. Glucose, insulin and C-peptide levels were determined to calculate the insulin sensitivity, the insulinogenic index, insulin levels at different time periods, 4 parameters of postprandial glycaemia and 9 model derived (Mari) parameters of beta-cell function, namely beta-cell glucoses sensitivity, rate sensitivity, potentiation factor ratios and insulin secretion rate in 5 different periods. All genetic analyses were carried out in Mx, a structural equation modeling program. In the univariate analyses the heritability of each variable was estimated individually. Subsequent multivariate analyses were performed to test overlap in the genetic factors influencing beta cell function, waist circumference and insulin sensitivity.
Results: The highest heritability was found for the insulinogenic index (63%), of which one third was shared with waist and insulin sensitivity. Beta cell glucose sensitivity had a heritability of 50% with a negligible overlap with genetic factors for waist and insulin sensitivity. Insulin secretion rate was only heritable before and during the first 2 postprandial hours (range 40-45%). Genetic factors determine half of the variability of the insulin sensitivity and of the postprandial glycaemic responses during mixed-meal tolerance tests. The fasting insulin but not postprandial insulin levels showed significant heritability (38%). In 7 beta cell function parameters, of which 5 were model-derived, genetic influence did not reach significance.
Conclusion: Classical and model derived beta cell function parameters of the first two hours of a meal test show a significant heritability. These parameters provide important physiological data that can be used to follow up results of gene finding studies.
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Genetic variability of G6PC2 influences beta cell function and insulin sensitivity in patients with newly diagnosed type 2 diabetes
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Background and aims: *G6PC2* (glucose-6-phosphatase catalytic subunit 2) is the catalytic subunit of glucose-6-phosphatase and it has specific expression in the beta cell. The rs560887 G allele of *G6PC2* is associated with higher levels of fasting plasma glucose (G) and increased first phase of insulin secretion during IVGTT. We evaluated the role of genetic variation at *G6PC2* in determining clinical and pathophysiological traits in patients with newly diagnosed type 2 diabetes (T2D).
Methods: 494 GAD-negative and drug-naïve patients (age 57.7±10.3 years, BMI 29.9±5.1 kg/m^2, HbA1c 7.0±1.4 %) with newly diagnosed T2D underwent standard clinical characterization. Furthermore, beta cell function (BF) and insulin sensitivity (SI) were assessed by mathematical modeling of G/C-

peptide curves during a 240' frequently sampled OGTT and by euglycemic insulin clamp, respectively. The beta-cell responses to the rate of increase of G (derivative or dynamic control: DC; median[IQ range]: 430[0.6-904] [pmol·m^{-2} BSA]/[mM·min^{-1}]) and to G concentration (proportional or static control, PC, presented as the insulin secretion rate at G concentrations of 5.5, 8.0, 11.0, 15.0 and 20.0 mM, respectively; 160±68, 222±121, 358±219, 569±378, 839±594 pmol·min^{-1}·m^{-2} BSA) are herein reported as measures of BF. SI is presented as the M value in the last 60' of the clamp (561[355-796] µmol·min^{-1}·m^{-2} BSA). We genotyped the following tag SNPs which reportedly capture ~95% of genetic variability of *G6PC2* locus: rs853770 , rs483109, rs12475700 (a), rs13387347 (b) and rs560887 (c). We report the results of variants a, b and c, because of their relevance to BF and SI.
Results: Both in our patients and in HapMap, a higher LD value (r^2= 0.54) was found between the proximal (a) and the distal (c) variant than between adjacent SNPs (a/b r^2= 0.10; b/c r^2= 0.29). Consistently with this observation, both major alleles of rs12475700 (A allele, frequency: 0.56) and rs560887 (G allele, frequency: 0.70) were associated with increased DC (A allele: +90±46, p<0.05; G allele: +95±49, p<0.03). In turn, the rs13387347 A allele (frequency: 0.43) was associated with reduced SI (-44±22, p<0.03), and increased levels of fasting C-peptide (+0.086±0.024 nmol/L, p=0.002) and insulin (+8.4±3.1 pmol/L, p<0.002). The increases in C-peptide/insulin were statistically significant also after adjustment for SI (p=0.006 and p<0.05 respectively).
Conclusion: In patients with newly diagnosed type 2 diabetes the *G6PC2* locus is associated with changes in BF and in SI of opposite sign, only partially explained with reciprocal compensation. These data could be of etiopathogenic and pathophysiological relevance and lead to clinical and therapeutic applications.
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Potential role of MTNR1B locus in regulating beta cell function and glucose levels in patients with newly diagnosed type 2 diabetes
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Background and aims: The high affinity melatonin receptor, *MTNR1B*, is expressed in brain, retina and endocrine pancreas. In non-diabetic subjects, the *MTNR1B* variant rs10830963 is associated with increased levels of fasting glucose (G), reduced beta cell function (BF) and increased risk of developing type 2 diabetes (T2D). We investigated the role of *MTNR1B* in determining clinical and pathophysiological traits in patients with newly diagnosed T2D.
Materials and methods: 494 GAD-negative and drug-naïve patients (age 57.7±10.3 years, BMI 29.9±5.1 kg/m^2, HbA1c 7.0±1.4 %) with newly diagnosed T2D underwent standard clinical characterization. Furthermore, beta cell function (BF) and insulin sensitivity (SI) were assessed by mathematical modeling of G/C-peptide curves during a 240' frequently sampled OGTT and by euglycemic insulin clamp, respectively. The beta-cell responses to the rate of increase of G (derivative or dynamic control: DC; median[IQ range]: 430[0.6-904] [pmol · m^{-2} BSA]/[mM · min^{-1}]) and to G concentration (proportional or static control, PC, presented as the insulin secretion rate at G concentrations of 5.5, 8.0, 11.0, 15.0 and 20.0 mM, respectively; 160±68, 222±121, 358±219, 569±378, 839±594 pmol · min^{-1} · m^{-2} BSA) are herein reported as measures of BF. SI is presented as the M value in the last 60' of the clamp (561[355-796] µmol · min^{-1} · m^{-2} BSA). We genotyped the following tag SNPs which reportedly capture ~94% of genetic variability of *MTNR1B* locus:: rs7102746 e rs11523890 upstream to, rs10830963 within and rs9666752 downstream to *MTNR1B*.
Results: The LD of these SNPs was found to be consistent with HapMap (CEU popolation). The rs11523890 G allele (frequency: 0.65) was associated with reduced DC (-136±55, p=0.002). The diabetes risk allele G of rs10830963 (frequency: 0.26) was significantly associated to reduced DC in a dominant model (GG+AG:-101±45, p<0.03), and also in multivariate analysis with rs11523890 as a covariate. In the combinatory rs11523890-rs10830963 genotype, no patient was homozygous for both risk alleles (GG-GG), but a tiny group (n=6, age:49±12; BMI:29.4±4.3) was homozygous for both non risk alleleles (CC-AA) and had the best DC (1982±642; p<0.01) and the worst SI (362±101; p<0.01). Finally, the rs9666752 A allele (frequency: 0.52) was associated with increased HbA_{1c} (+0.22±0.09%, p<0.02).
Conclusion: *MTNR1B* may play a relevant role in the regulation of G levels and BF in patients with newly diagnosed T2D. These data, if confirmed. could lead to clinical and therapeutic application, including, but not limited to, identification of specific subgroups of patients for diagnostic-therapeutic purposes.
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Pancreatic islets of melatonin receptor knock-out mice
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Background and aims: Genome-wide-association studies (GWAS) have recently suggested that a receptor (MTNR1B) for the pineal hormone melatonin is involved in the pathogenesis of Type 2 Diabetes Mellitus (T2DM). Melatonin binds predominantly to its receptors MTNR1A (MT1) and MTNR1B (MT2), which are G-protein coupled receptors and are proposed to exert an inhibitory effect on insulin secretion. Here, we investigate whether a metabolic phenotype evolves in mice when melatonin receptor signalling is disrupted. Also, we examined whether a knock-out of melatonin receptors affects islet morphology and function.
Materials and methods: Knock out mice for MT1, MT2 and both receptors were kindly provided by Professor David Weaver. Every second week blood samples were taken from the Saphenous vein in awake male mice. We measured glucose and insulin concentrations in plasma. We also performed immunohistochemistry to calculate β-cell mass and area and to determine cellular location of the receptors in islets. Standard errors are given as standard deviations.
Results: Plasma glucose (8.61 ±1.43 mM) and insulin levels (1.58 ±0.98 ng/ml) did not differ between the mouse lines and did not change over time (10-22 weeks). MT2 knock-out mice were significantly heavier than WT, MT1 and MT1/2 knock-out mice (MT2: 39.7 ±1.0g; WT: 34.0 ±0.9g; MT1: 31.0 ±0.7g; MT1/2: 30.45g ±0,7). Mean β-cell mass did not vary between mouse lines, but there was a trend towards increased islet area in MT2 and MT1/2 double knock-out mice (MT2: 2.42 x 10^{-5} ±2.0 x 10^{-5} AU (Arbitrary Units); MT1/2: 2.44 x 10^{-5} ±1.7 x 10^{-5} AU; WT: 1.52 x 10^{-5} ±1.1 x 10^{-5} AU; MT1 1.39 x 10^{-5} ±1.0 x 10^{-5} AU).
Conclusion: Our data indicate differences in whole body metabolic regulation in MT2 receptor knock-out mice, since they are significantly heavier than WT, MT1 and MT1/2 knock-out mice. The trend towards increased islet area in MT2 and MT1/2 double knock-out mice suggests that MT2 and MT1/2 knock-out mice tend to have more islets than MT1 and WT mice, which could be explained by increased demands for insulin in the heavier mice.

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Effects of KCNQ1 on glucose-induced insulin secretion in rat pancreatic beta cells or on glucagon-like peptide-1 secretion in cultured NCI-H716 cells
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Background and aims: Two genome-wide association studies conducted in Japanese populations have identified a gene encoding *KCNQ1* (potassium voltage-gated channel, KQT-like subfamily, member 1) as a strong susceptibility gene to type 2 diabetes, and the polymorphisms in the *KCNQ1* have been also shown to be associated with ß cell functions, or with secretion of glucagons-like peptide-1 (GLP-1). The aim of the present study is to know the role of the KCNQ1 in insulin secretion using isolated rat pancreatic ß cells, or in GLP-1 secretion using cultured human GLP-1 secreting cell lines (NCI-H716).
Materials and methods: We examined the expression of *KCNQ1* mRNA by real-time PCR. The rat isolated ß cells were incubated with or without choromanol 293 (specific KCNQ1 inhibitor), and insulin secretion from the cells were measured by enzyme linked immunosorbent assay (ELISA). We also measured GLP-1 secretion from the NCI-H716 cells with or without the treatment of chromanol 293 by ELISA.
Results: We found clear expressions of the *KCNQ1* as well as *KCNE2*, a ß subunit of the potassium channel, in isolated rat pancreatic islets by RT-PCR. Treatment of isolated rat ß cells with chromanol 293B (100 µM) could signifi-

cantly increase the glucose (16.7mM)-induced insulin secretion. A similar effect could be observed in isolated pancreatic ß cells under the presence of 300 µM of tolbutamide, although the difference was not statistically significant. The KCNQ1 inhibitor did not affect insulin secretion from the ß cells under low glucose conditions or under the presence of 30 mM KCl. In NCI-H716 cells, the expressions of *KCNQ1* and *KCNE1 - 5* were detected. The treatment with chromanol 293 increased bethanechol (1000 µM)-induced GLP-1 secretion, but did not affect GLP-1 secretion under a basal condition or under the presence of 10 µM ionomycine.
Conclusion: These results suggest that KCNQ1 can regulate secretion of insulin and GLP-1, and may contribute to the susceptibility to type 2 diabetes by decreasing glucose-induced insulin secretion in the pancreatic β cells or diet-induced GLP-1 secretion in the L cells.

Effects of KCNQ1 inhibitor on insulin secretion in pancreatic beta cells, or on GLP-1 secretion in NCI-H716 cells.

Insulin secretion	Mean ± sem	ng/hr/mg protein		
chromanol 293B (100 µM)	2.8 mM glucose	16.7 mM glucose	Tolbutamide 300 µM	30 mM KCl
vehicle	66.1 ± 4.6	386.0 ± 74.5	465.3 ± 65.5	745.6 ± 66.7
+	66.1 ± 3.1	608.8 ± 100.9	627.6 ± 85.9	831.7 ± 43.1
		P < 0.05	P < 0.1	NS
GLP-1 secretion	Mean ± sem	pg/hr/mg protein		
chromanol 293B (100 µM)	basal	Meat hydrolysate 0.5%	Betanechol 1000 µM	Ionomycine 10 µM
vehicle	4029 ± 328	13398 ± 131	8701 ± 385	20940 ± 1545
+	3738 ± 107	16483 ± 513	17222 ± 1103	21323 ± 836
		P = 0.15	P < 0.0001	NS

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Polymorphisms in CACNA1D affect insulin release and channel expression and associate with type 2 diabetes
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Background and aims: Voltage-gated Ca^{2+} channels of the L-type are essential triggers for insulin secretion in rodents but little is known about their role in human type 2-diabetes. We here set out to determine the contribution of the human L-type channel subtype $Ca_v1.3$ (encoded by CACNA1D) for inherited capacity of insulin secretion.
Materials and methods: *Gene expression in human islets:* Total RNA was isolated from pancreatic islet donors including 45 non-diabetics / 6 diabetics (24/4 males, 21/2 females, age=56±10/58±15, BMI=26±3/28±4) using the AllPrep DNA/RNA Mini Kit (Qiagen) and analyzed using Gene 1.0 ST whole transcript based assays (Affymetrix). *Genetic studies:* DNA from the Diabetic Genetics Initiative was analysed with the Affymetrix Human Mapping 500K GeneChip® in order to identify single nucleotide polymorphisms. Three candidate SNPs were tested in 766 non-diabetics (358 males, 408 females, age=49±13, BMI=25±4) from the Botnia study by genotyping using Taqman allelic discrimination assays and common variants were detected by ABI PRISM 7900. Phenotypes were assessed with oral (OGTT) or intravenous (IVGTT) glucose tolerance tests with insulin measured using a radioimmunoassay. Insulin phenotypes were corrected for age, gender and BMI.
Results: In non-diabetics, the marker rs312480 associated significantly with inappropriate fasting insulin release (IVGTT, corrected insulin response (CIR), p=0.027, additive model) and in individuals younger than 41 years significantly affected the acute insulin (AI) release (OGTT, p=0.009) as well as altered levels at time points 60 and 120 minutes (p=0.006 and 0.032 respectively). This marker also evoke our interest as it is located within the 5'UTR region of the gene, contains putative methylation sites and significantly affects expression of the gene (p=0.014) in our microarray study. The marker rs312486 was found to correlate with increases in 1- and 2-h glucose levels (OGTT, p=0.017 and 0.046 resp.). In a larger material (Botnia-PPP, 5210 individuals) this polymorphism showed significant association with type-2 diabetes status (p=0.016, OR=1.317). Interestingly, the minor allele of this marker links significantly with raised blood pressure in younger individuals (<41 years). The third SNP of interest, rs9841978, was found to relate to reductions in first phase insulin secretion (p=0.001). In addition, our microarray data suggests an association between the expression of CACNA1D and the diabetes status (p=0.025).
Conclusion: Single nucleotide polymorphisms in the $Ca_v1.3$ encoding gene CACNA1D affect mRNA expression of the channel in human pancreatic islets and associate with impaired insulin secretion as well as type-2 diabetes.
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A common variant in the *PAX6* gene influences islet function in man
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Background and aims: PAX6 is an important regulator of pancreas development and a key transcription factor for genes involved in glucose homeostasis, including insulin, incretins and prohormone convertase genes. Impaired glucose tolerance and insulin secretion has been reported in families with protein disrupting *PAX6* mutations and suggested to result from defective proinsulin processing due to lack of *PCSK1*. In this study we investigated the effect of a common *PAX6* variant on glucose homeostasis and insulin processing as well as expression of target genes.
Materials and methods: A candidate SNP was identified in a genome-wide association study. Association with glucose tolerance, insulin processing and secretion was assessed in four Scandinavian cohorts. Insulin secretion and expression of *PAX6* and transcriptional targets was studied in human pancreatic islets.
Results: We identified a SNP that was associated with fasting proinsulin/insulin ratio in the Diabetes Genetics Initiative (DGI) genome-wide association scan. The G allele of rs685438 was associated with lower fasting proinsulin/insulin ratio as well as with increased fasting insulin (p=0.001) and HOMA-IR (p=0.0008). Expression of *PAX6* (p=0.01) and *PCSK1* (p=0.001) was lower in pancreatic islets from human donors carrying the G-allele. The effect on fasting proinsulin/insulin ratio was also seen in the Helsinki birth cohort study (P=0.07), as was higher fasting insulin (p=0.04) and HOMA-IR (p=0.03). In the Botnia Prospective study G allele carriers had higher 2 hour insulin levels (p=0.03) and higher 2 hour glucose levels (p=0.0001). Acute arginine-stimulated insulin secretion in a cohort of 167 diabetic and non-diabetic individuals was reduced (p=0.02). Glucose stimulated insulin secretion was also significantly lower in human pancreatic islets (p=0.002). Further, G allele carriers had lower fasting plasma levels of both GIP (p=0.03) and glucagon (p=0.03).
Conclusion: A common variant in *PAX6* affects *PAX6* and *PCSK1* expression and islet function. In spite of increased insulin resistance individuals with low expression have lower proinsulin/insulin ratio rather than higher, which was previously reported for carriers of *PAX6* mutations. In contrast, they have reduced acute arginine-stimulated insulin secretion, a measure of total insulin secretion capacity, and reduced incretin levels, which is in accordance with the expected effect of PAX6 on expression of insulin and incretin mRNA.

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TCF7L2-conferred apoptosis in pancreatic beta cells involves the p53 pathway
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Background: The SNP rs7903146 of the TCF7L2 gene increases risk for type 2 diabetes through a perturbed insulin secretion, an impaired incretin effect and impaired cell survival (increased apoptosis and/or decreased proliferation). The mechanisms by which TCF7L2 would influence beta cell apoptosis and/or proliferation are not known.
Aim: To identify the molecular mechanism/s by which TCF7L2 confers the apoptotic effect in pancreatic beta cells.
Material and methods: Chromatin immunoprecipitation on microarray (ChIP-on-chip) was used to identify potential TCF7L2 target genes in INS-1

832/13 cells. The expression level of *Tcf7l2* and *Tp53inp1* in INS-1 832/13 cells was manipulated using siRNA and measured using qPCR. Apoptosis was measured using antibodies against Annexin V, and 7-AAD and visualized using confocal microscopy.
Results: The p53 pathway was identified as a key TCF7L2 target using ChIP-on-chip technology. SiRNA of *Tcf7l2* was subsequently used to verify the ChIP-on-chip results and a knock down of *Tcf7l2* (69.5 ± 0.04%) in INS-1 832/13 cells results in elevated *Tp53* (19.2 ± 0.05%) and *Tp53inp1* (52.6 ± 0.23%) mRNA levels. Interestingly, *TP53INP1* has recently been associated with increased risk of T2D. Upregulation of *Tp53* and *Tp53inp1* was associated with increased apoptosis in the beta cell line (3.92 ± 1.68 fold). *Tp53inp1* is a co-activater of Homeodomain interacting protein kinase 2 (HIPK2), which can specifically phosphorylate p53 on serine 46 and thereby induce apoptosis. A rescue experiment restoring (decreasing) the *Tp53inp1* expression level prevented the increase in apoptosis seen after *Tcf7l2* knock down, suggesting that the *Tcf7l2* effect required the p53 pathway and *Tp53inp1*.
Conclusion: The p53 pathway, particularly TP53INP1 seems to be central for mediating effects of TCF7L2 on apoptosis in beta cells. We thereby provide a link between two genes shown to increase risk of T2D, *i.e. TCF7L2* and *TP53INP1*.
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Progression of beta cell dysfunction in Japanese type 2 diabetic patients in comparison with those who carry S20G mutation of islet amyloid polypeptide gene: a long-term follow-up study
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Background and aims: Type 2 diabetic patients have islet amyloid deposition with considerably high frequency. Islet amyloid polypeptide (IAPP) was identified as a main constituent of the islet amyloid, and has been considered to be associated with pathophysiology or development of human type 2 diabetes due to its amyloidgenicity. We found S20G (AGC^{Ser} to GGC^{Gly}) mutation of *IAPP* gene, and have investigated that the G20-IAPP variant had stronger amyloidogenicity and cytotoxicity than wild type IAPP *in vitro*. However, there is little clinical data how impairments of insulin secretion progress in patients carrying S20G mutation. The long time course of the beta-cell function in type 2 diabetic patients also remains unknown. In this study, we first estimated the decline of insulin secretion in non-obese (BMI < 30 kg/m^2) Japanese type 2 diabetic patients without S20G mutation (T2D-patients), and then compared it with that of type 2 diabetic patients carrying S20G mutation (S20G-patients).
Materials and methods: We studied 70 T2D-patients and 6 S20G-patients. Patients with renal dysfunction (serum creatinine > 1.1 mg/dl) or those carrying anti-insulin antibodies were excluded. Serum C-peptide (CP) was employed as the indices for evaluation of endogenous insulin secretion, fasting level (F-CP), 5 min value after intravenous 1 mg glucagon injection (5'-CP), and 5 min increment from the basal after glucagon injection (d-CP). CP was measured by immunoenzymometric assay. The individual annual decline of endogenous insulin secretion (IAD) was calculated from the individual regression line between CP and duration (years after diagnosis). The estimated annual decline of endogenous insulin secretion (EAD) was calculated from regression line between total points of CP and duration (n=527 for F-CP, n=165 for 5'-CP and d-CP) in all T2D-patients (n=70), although it was a cross-sectional analysis. In T2D-patients, we used EAD of both 5'-CP and d-CP instead of IAD, because IAD of both 5'-CP and d-CP could not be calculated due to small number of the plots of each patient.
Results: IAD of F-CP was greater in S20G-patinets than T2D-patients (0.140±0.076 Vs 0.034±0.110 ng/ml/year, p=0.025). In T2D-patients, EAD was 0.031 ng/ml/year and it was almost same as IAD. IAD of both 5'-CP (n=4) and d-CP (n=4) in S20G-patients were high compared with those of EAD in T2D-patients (5'-CP: 0.202±0.096 Vs 0.101 ng/ml/year and d-CP: 0.118±0.056 Vs 0.062 ng/ml/year, respectively).
Conclusion: We showed the long-termed decline of the endogenous insulin secretion in non-obese Japanese T2D-patients. The results suggested that the decline of the endogenous insulin secretion is more rapid in S20G-patients than T2D-patients.

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A common polymorphism in the ccl2 gene regulatory region affects mcp-1 gene expression and function of isolated non-diabetic and type 2 diabetic islets
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Background and aims: It has been shown that human islets release monocyte chemoattractant protein-1 (MCP-1), a macrophage chemokine which may impair the fate of the islets in vivo and after a transplant. In this study we evaluated presence and role of monocyte chemoattractant protein-1 (MCP-1) and its receptor CC -chemokine receptor-2 (CCR2) in isolated non-diabetic (Ctrl) and type 2 diabetic (T2DM) islets, including genotyping for a common polymorphism.
Materials and methods: Pancreatic islets were obtained from 47 Ctrl and 18 T2DM multiorgan donors. We evaluated glucose-stimulated insulin release (IR, μU/islet/min, expressed as Stimulation Index: SI), measured MCP-1, CCR2 and insulin mRNA expression, and analyzed the MCP-1 (-2518 G/A, rs1024611 in the promoter region) and the CCR2 (G46295A Val64Ile, rs1799864 in exon2) Single Nucleotide Polymorphisms (SNP).
Results: As expected, in T2DM islets SI (1.44±0.17) and insulin gene expression (7.15±1.5) were significantly reduced respect to Ctrl (2.34±0.20 and 26.47±6.64, respectively) islets (all p<0.001). MCP-1 and CCR2 expression was significantly higher in T2DM (3.80±0.62 and 0.31±0.05, respectively), respect to Ctrl (1.60±0.18 and 0.062±0.02, respectively) islets (all p<0.001). We observed a positive significant correlation between MCP-1 and CCR2 gene expression (R2=0.235, p=0.0032), a negative significant correlation between MCP-1 mRNA expression and both SI (R2=0.116, p=0.0095) and IR at 16.7 mmol/l glucose (R2=0.077, p=0.037), and a positive significant correlation between MCP-1 gene expression and BMI (R2=0.076, p=0.039). Both in Ctrl and in T2DM, we found that MCP-1 mRNA expression was significantly higher in CC and CT respect to TT genotype groups, with a SI significant lower in CC respect to TT genotype group. Intriguingly, islets presenting both SNPs studied (rs1799864 and rs1024611), showed a high MCP-1 and CCR2 gene expression and a reduced beta-cell function.
Conclusion: These data show that MCP-1 gene is present in human pancreatic islets, it's more expressed in T2DM respect to Ctrl islets, is regulated by the -2518 G/A polymorphism, and correlates with glucose-stimulated insulin release. The study of MCP-1 expression and genotype could be useful also in understanding the inflammatory response in vivo against pancreatic islets after transplantation.

PS 5 Candidate genes in type 2 diabetes

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Relationship between adiponectin and insulin-like growth factor-binding protein 1 and their combined effects in type 2 diabetes

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Background and aims: Evidence has demonstrated that serum levels of adiponectin (AdipoQ) and insulin-like growth factor-binding protein 1 (IGFBP-1) are decreased in type 2 diabetes (T2D) patients compared to non-diabetic control subjects. *AdipoQ* genetic polymorphisms are found to be associated with T2D. *IGFBP-1* genetic polymorphisms are associated with impaired renal function in T2D. However, whether adiponectin and IGFBP-1 have gene-gene and protein-protein interactions in T2D is unknown. In the present study, we attempted to explore possible relationship between these two molecules and their effects in T2D.
Materials and methods: We genotyped five SNPs (-11426A/G, -11391G/A, -11377C/G, +45T/G Gly15Gly and +276A/C) in the *AdipoQ* gene and two SNPs (-575G/A and +4403A/G Ile253Met) in the *IGFBP-1* gene in 163 Swedish T2D patients. Of the patients, 85 had family history of diabetes (FHD). We also measured serum levels of adiponectin and IGFBP-1 in all patients by using radio-immunoassays. We further employed generalized multifactor dimensionality reduction (GMDR) to assess the impact of gene-gene interactions (the patients without FHD as controls). Linear regression and logistic regression models were used for correlation analyses of adiponectin and IGFBP-1 serum levels and for confirmation of the data from GMDR analyses.
Results: We found that serum adiponectin levels in T2D-FHD(+) (4.47 mg/l, geometrical means 4.07-4.90) was significantly lower compared to T2D-FHD(-) (5.50 mg/l, 4.90-6.17) (P=0.006). GMDR analyses of all studied SNPs between the *AdipoQ* and *IGFBP-1* genes implicated that two promoter polymorphisms -11377C/G in the *AdipoQ* gene and -575G/A in *IGFBP-1* had an impact of gene-gene interaction (P=0.054, cross-validation consistency 10/10 and testing accuracy 59.9%). Further analyses indicated that there was a significant correlation between adiponectin and IGFBP-1 at protein levels in T2D-FHD(-) (R=0.268, P=0.023) but not in T2D-FHD(+) (P=0.167). Among T2D-FHD(-) carrying with GG and CG genotypes of *AdipoQ* -11377C/G polymorphism, this correlation between adiponectin and IGF-BP1 was remained (R=0.414, P=0.023) but not presented in T2D-FHD(-) with CC genotype (P=0.295). Similarly, serum levels of adiponectin and IGFBP-1 were significantly correlated (R=0.303, P=0.047) in T2D-FHD(-) carrying with AA and GA genotypes of IGFBP1 -575G/A polymorphism, but not in T2D-FHD(-) with GG genotype (P=0.374).
Conclusion: Data from the present study implicate that promoter polymorphisms of the *AdipoQ* and *IGFBP-1* genes may have an impact of gene-gene interaction in T2D. These two promoter polymorphisms may influence the correlation between adiponectin and IGFBP-1 at protein levels. Replication study with additional T2D patients and non-diabetic control subjects will be conducted to further understand genetic and functional effects of adiponectin and IGFBP-1 in the development of T2D.
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Circulating HMW adiponectin is positively correlated and shares a common genetic background with urinary albumin excretion in non diabetic white caucasians from Italy

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Background and aims: Circulating levels of adiponectin, an insulin sensitizing hormone, have been reported to be paradoxically increased in patients with higher urine albumin excretion (UAE), a condition characterized by insulin resistance. In order to avoid the possible confounding effects exerted in these previous studies by the presence of diabetes - and related treatments - and that of reduced kidney function, we investigated the relationship between adiponectin and UAE levels in a large (n = 640, 246M/394F), family-based sample of relatively young (age 40.3 ± 14.5 yrs) non diabetic, White Caucasians from Italy without known kidney impairment who were not on any pharmaceutical treatment.
Materials and methods: UAE was measured by nephelometric method and reported as urinary albumin-creatinine ratio (ACR). Serum adiponectin (high, medium and low molecular weight isoforms; HMW, MMW, LMW) levels were measured by ELISA. Glomerular filtration rate was estimated by the reciprocal of serum cystatin C (CC) expressed in mg/L multiplied by 100 (CC-GFR; mean: 125.4 ± 35.9). Five SNPs in the *ADIPOQ* gene, previously reported to be associated to adiponectin levels and/or diabetic nephropathy (rs182052, rs17300539, rs2241766, rs1501299 and rs677395) were genotyped. A linear mixed effects model was used to assess both phenotypic correlations and to test associations. Bivariate analyses were conducted to study genetic correlations between adiponectin isoforms and UAE.
Results: ACR levels (median: 0.53 mg/mmol; range 0.06 - 12.4) were directly associated with HMW adiponectin ($\beta \pm SE = 0.058 \pm 0.02$, $p = 3.7 \times 10^{-3}$). Further adjustment for CC-GFR, did not significantly change this association ($\beta = 0.06 \pm 0.02$, $p=2.7 \times 10^{-3}$). A significant genetic correlation (rhog = 0.38 ± 0.20; p=0.04) was observed between ACR and HMW adiponectin. Among the *ADIPOQ* SNPs tested, rs17300539, which was associated with both HMW ($p = 4.4 \times 10^{-5}$) and ACR ($p=2.7 \times 10^{-3}$), partially accounted for this genetic correlation (2.5%).
Conclusion: In conclusion, circulating HMW adiponectin and UAE levels are directly correlated and share, at least partly, a common genetic background, involving the *ADIPOQ* locus for a small proportion of it.
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Associations of common variants of ADIPOQ, ADIPOR1 and ADIPOR2 with adiponectin concentration and diabetes incidence in the Diabetes Prevention Program

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In the Diabetes Prevention Program, a trial enrolling people from multiple ethnic backgrounds who were overweight and had impaired glucose regulation, baseline concentrations of adiponectin were independently predictive of incident type 2 diabetes. We examined the association of genetic variation in the genes encoding adiponectin (*ADIPOQ*) and the two known adiponectin receptors (*ADIPOR1*, *ADIPOR2*) with circulating adiponectin concentrations and with diabetes incidence. Fourteen of 24 *ADIPOQ* SNPs were nominally associated with adiponectin concentrations; 9 exceeded experiment-wide significance and 4 exceeded genome-wide significance in the entire study population (rs1648707 $p=10^{-15}$; rs17366568 $p=10^{-14}$; rs6810075 $p=10^{-11}$; rs182052 $p=10^{-12}$). For these 4 SNPs, minor allele homozygotes had 11-25% lower mean circulating concentration of adiponectin than major allele homozygotes. Among white subjects only (n=1622) these SNPs showed similar patterns of association (rs1648707 $p=10^{-11}$; rs17366568 $p=10^{-14}$; rs6810075 $p=10^{-9}$; rs182052 $p=10^{-10}$), indicating that these signals are not confounded by population stratification. One *ADIPOR1* SNP (rs10800890) was also associated with adiponectin concentrations ($p=10^{-4}$); no *ADIPOR2* SNPs were associated with adiponectin concentrations. Three of 22 *ADIPOR1* SNPs and 2 of 31 *ADIPOR2* SNPs were associated with diabetes incidence in the whole population ($p=10^{-2}$ to 10^{-3}). None of the *ADIPOQ* variants, and specifically none of those associated with adiponectin concentrations, was associated with diabetes incidence in the whole study population. Two of the 77 evaluated SNPs interacted with treatment to influence diabetes incidence in the whole study population (*ADIPOQ* rs17373414 p=0.04, hazard ratio higher in lifestyle than placebo; *ADIPOR2* rs758027 p=0.01, hazard ratio higher in metformin than placebo). A parallel rs758027 interaction was evident among whites, again arguing against confounding by population stratification. *ADIPOQ* SNPs are significantly associated with adiponectin concentrations in the DPP cohort, confirming the results published from cohorts with lower

diabetes risk and expanding them to a multi-ethnic population with impaired glucose regulation. Despite associations with circulating adiponectin concentrations and the known robust relationship between adiponectin concentrations and diabetes risk in this cohort, loci influencing diabetes risk did not overlap with those influencing adiponectin concentrations in the DPP. This highlights the complex relationships of genetic and non-genetic determinants of adiponectin concentrations with type 2 diabetes risk.
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Effect of variants in the RORA gene on risk of type 2 diabetes
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Background and aims: A genetic variation in the RAR-related orphan receptor alpha (ROR-α) gene (*RORA*) was found to be associated with reduced insulin secretion in the DGI genome wide association study for early insulin secretion. ROR-α has been previously implicated in glucose and lipid metabolism. The aim of the study is to validate the effect of genetic variants in *RORA* on insulin secretion, and to explore its effects on risk for type 2 diabetes (T2D) and related phenotypes.
Materials and methods: Six *RORA* variants (rs10519116, rs11071557, rs17204545, rs2414689, rs4774389 and rs4775292) were genotyped in three cohorts, totaling 13,560 Scandinavian individuals (Malmö Case Control [MCC], n=6,380; Prevalence, prediction and prevention of diabetes in Botnia [PPP], n=4,852, and Botnia Prospective Study [BPS], n=2,328 with a median follow-up period of 7.6 years).
Results: In the DGI, the T-allele of rs4775292 was associated with reduced insulin secretion (N=1,018, beta (sem) -0.214 (0.041), P=0.0024). Furthermore, in the independent follow-up PPP study the same allele showed increased insulin secretion in young (N=1,499, 0.103 (0.031), P=0.00079), but decreased in elderly (N=1,245, -0.081 (0.030), P=0.0073, median age 50 yrs as cut-off). Combined analysis confirmed association of the T-allele of rs4775292 with reduced insulin secretion in elderly individuals (N=2,698, -0.075 (0.023), P=0.0011). In the MCC, in line with increased insulin secretion, the T-allele of rs4775292 was associated with protection from T2D (OR [95%CI]: 0.78 [0.61-0.99], P=0.039) in young, but no effect was seen (1.00 [0.91-1.11], P=0.94) in elderly. Of note, in the MCC, the C-allele of another SNP, rs10519116, was more frequent in cases than in controls (26.5% vs. 24.2%, P=0.015). This difference was more pronounced in elderly individuals (27.0% vs. 24.0%, P=0.0027), which translated into an age, sex and BMI adjusted OR for T2D of 1.16 [1.03-1.29], P=0.011. The same C-allele of rs10519116 and also the T-allele of rs4775292 were associated with increased 2hr proinsulin and GIP levels during OGTT (rs10519116: 0.081 (0.036), P=0.024 and 0.119 (0.037), P=0.0014; rs4775292: 0.080 (0.030), P=0.0079 and 0.105 (0.031), P=0.00091) in male PPP participants. Furthermore, the same T-allele of rs4775292 and the GT/TT-genotype of rs11071557 were associated with decreased HDL and APOA1 both at baseline (rs4775292: -0.037 (0.014), P=0.0087 and -2.77 (1.00), P=0.0059; rs11071557: -0.183 (0.057), P=0.0015 and -14.43 (4.08), P=0.00042) and at follow-up (rs11071557: -0.203 (0.075), P=0.0071 and -11.17 (4.98), P=0.025) in male BPS participants. Finally, two SNPs were associated with decreased insulin sensitivity (rs17204545: PPP -0.053 (0.020), P=0.0079 and rs2414689: BPS -0.077 (0.026), P=0.0026) in male participants. Interestingly, *RORA* shows a rich methylation pattern where rs4775292 is a candidate for a CpG site. Methylation studies are ongoing to provide insights whether they can explain the age effect on insulin secretion and risk of T2D.
Conclusion: These results suggest that genetic variants in the *RORA* gene are associated with increased risk of T2D, and influence glucose and lipid metabolism.
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Vaspin is involved in the pathophysiology of type 2 diabetes by regulating insulin sensitivity
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Background and aims: Visceral adipose tissue derived serine protease inhibitor (vaspin) is a novel adipokine that may link obesity, insulin resistance (IR) and type 2 diabetes (T2D), but so far its pathophysiological role remains largely unknown. The first aim was to study the effects of recombinant vaspin treatment on insulin sensitivity in *db/db* mice. In addition, we investigated the role of genetic variation in the human vaspin gene in the pathogenesis of T2D.
Materials and methods: Animal studies: After recombinant vaspin administration (1mg/kg body weight i.p.; at 6 pm and at 6 am prior to the tests), we performed glucose tolerance tests (2g/kg body weight i.p.) and hyperinsulinemic-euglycemic clamps in *db/db* mice (N=5 for each test). Human genetic studies: *Vaspin* (exons, exon-intron boundaries, 5′ and 3′ UTRs) was sequenced in DNA samples from 48 unrelated Caucasian subjects (ABI PRISM 3100 Avant; Applied Biosystems Inc.). Six single nucleotide polymorphisms (SNPs) identified by sequencing and 22 haplotype tagging SNPs representative for their linkage disequilibrium groups (r^2>0.8 and minor allele frequencies >0.05) were genotyped in 1046 clinically well-characterized Sorbs from Germany for subsequent association studies on metabolic traits including insulin resistance and secretion indices (e.g. fasting Belfiore, Stumvoll index, HOMA-IR) based on glucose tolerance test in non diabetic subjects. *P* values <0.05 were considered to be of nominal statistical significance. For *in vitro* analyses of the effects of the stop codon mutation (p.R211X), full-length (wild type) and short-length (carrying the mutation) *vaspin* was cloned into p3xFLAG-myc-CMV™ expression vector (Sigma-Aldrich) and transfected into HEK-cells (Fugene® HD Transfection Kit; Roche). Proteins were detected by western blot.
Results: Animal studies: Vaspin administration in *db/db* mice resulted in improved glucose tolerance (P<0.05). Consistently, glucose infusion rate (GIR) during the steady state of the clamp significantly increased after vaspin treatment (P<0.05). Human genetic studies: Sequencing of the vaspin gene revealed one SNP (rs61757459) in exon 3 resulting in a STOP-codon (p.R211X). Western blot experiments showed that full-length and short-length vaspin were expressed in eukaryotic cells. Short-length vaspin yielded in a prominent ~25-kDa band. Several SNPs were nominally associated with WHR (waist-to-hip-ratio), 30min glucose levels or 2-hr-insulin levels (adj. for age, sex and BMI). Furthermore one SNP (rs2236242) showed additional associations with $AUC_{Glucose}$, insulin sensitivity and insulin resistance indices.
Conclusion: In conclusion, our data demonstrate the substantial insulin sensitizing effect of vaspin and suggest a role of *vaspin* genetic variants in the pathophysiology of insulin resistance and T2D.
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ABO blood groups and incidence of type 2 diabetes in men and women
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Background and aims: Previous small studies associated ABO blood types with the prevalence of diabetes. Recent genome-wide scans identified *ABO* locus might determine various risk factors for type 2 diabetes.
Materials and methods: We prospective examined the relationship between ABO blood types and the risk of incident type 2 diabetes in men and women from two cohorts: the Health Professionals Follow-up Study (HPFS) and the Nurses' Health Study (NHS).
Results: In the HPFS, during 452,404 person-years of follow-up, 1,764 participants developed type 2 diabetes. Compared with participants with blood group O, the relative risk (RR) associated with blood group A, B, and AB were 1.08 (95% confidence interval [CI] 0.97-1.20), 1.15 (1.00-1.34), and 1.31 (1.10-1.57). In the NHS, during 111,7247 person-years of follow-up, 4,376 participants developed type 2 diabetes. Compared with participants with blood group O, the RRs (95% CI) of type 2 diabetes associated with blood groups A, B, and AB were 1.10 (1.03-1.18), 1.03 (0.94-1.13), and 1.02 (0.91-

1.15), adjusting for traditional risk factors. The association of blood group A with diabetes risk was more evident in postmenopausal than premenopausal women. Overall, as compared with O blood group, the non-O blood group (blood group A, B, or AB) was associated with 12% (2-24%) and 7% (1-14%) increased diabetes risk in men and women; respectively; and was associated with 9% (4-15%) increased diabetes risk in the combined samples. The population attributable risk (PAR) for type 2 diabetes due to non-O blood group was 4.7%.
Conclusion: ABO blood types were significantly associated with the risk of type 2 diabetes. Non-O blood groups were associated with an increased risk of diabetes in both genders. The associations of specific blood groups with diabetes risk showed a gender difference.

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Contribution of mother and father in the familiar transmission of type 2 diabetes mellitus according to the proband's gender
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Background and aims: Type 2 diabetes mellitus (T2DM) shows strong familiar aggregation: the relative role of maternal and paternal familiarity, however, needs to be further investigated. In this study, we evaluated the distinct contribution of the mother and the father in T2DM transmission according to proband's gender.
Materials and methods: We evaluated through a structured interview the familial transmission in 1076 T2DM patients aged <70 years attending our Diabetes Clinic, divided into two groups according to the age at diagnosis: 30-49 years (Group A: 540 consecutive patients, M/F: 366/174) and 50-69 years (Group B: 536 consecutive patients, M/F: 312/224).
Results: Group A showed the following parental transmission: only mother: 34.6%; only father: 19.8%; both parents: 10.7%. Thus, mother was affected in 45.3%, father in 30.5% (p<0.0001). When proband's gender was considered, parental transmission was: a) in men (n=366): only mother: 34.2%; only father: 22.4%; both parents: 7.7%. Thus, mother was affected in 41.9%, father in 30.1% (p<0.018); b) in women (n=174): only mother: 35.6%; only father: 14.4%; both parents: 17.2%. Thus, mother was affected in 52.8%, father in 31.6% (p<0.004). Thus, the excess of maternal transmission was present in both genders, but women probands showed an increased probability to have both parents affected (p=0.026); furthermore, in the presence of father affected, the conditional probability to have also mother affected was greater in women than in men (55% vs 25%, p=0.007). Familiarity was not modified by proband's body mass index. In Group B, parental transmission was less frequent than in Group A, mother being affected in 35.7% vs 45.3% in Group A (p=0.023), father in 14.8% vs 30.5% in Group A (p<0.0001). Also in Group B, however, mother was more frequently affected than father (p<0.0001). By considering the two groups together, multivariate linear regression showed that, in comparison to the absence of familiarity in parents: a) in men probands, familiarity in the mother anticipated diabetes diagnosis 2.6±0.7 yrs (p<0.0001), in the father 5.1±0.9 yrs (p<0.0001) and in both parents 9.1±1.4 years (p<0.0001); b) in women probands, familiarity in the mother anticipated diabetes diagnosis 2.2±1.0 yrs (p=0.023), in the father 2.5±1.3 yrs (p=0.065) and in both parents 7.2±1.5 yrs (p<0.0001). Thus, familiarity in the father anticipated the diagnosis of T2DM more strongly in men than in women. Men excess in both Group A and Group B induced us to compare our data with the percentage of men in T2DM patients aged < 70 years living in the District in which our Hospital is located, obtained from the Health Service data of our Region: this percentage was 65% (vs 68% in Group A) in patients with diabetes diagnosis at age 30-49 years and 57% in those with diabetes diagnosis at age 50-69 years (vs 58% in Group B). Thus, an excess of diabetic men vs women was present in our District, independently from attendance at our Diabetes Clinic.
Conclusion: In Type 2 diabetes: i) familiarity predicts early diagnosis; ii) maternal transmission is stronger than the paternal-one in both proband genders independently of age at diagnosis; iii) if father is affected, maternal co-transmission is more frequent in women.
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***TCF7L2* and therapeutic response to sulfonylureas in patients with type 2 diabetes**
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Background and aims: Variants in the *TCF7L2* have been shown to be associated with an increased risk for type 2 diabetes (T2D). Since the association with diabetes could be explained by effects on insulin secretion, we investigated whether patients with diabetes risk alleles at rs7903146 might have an altered hypoglycaemic response to sulfonylureas (SUs).
Materials and methods: We recruited 189 patients with T2D being treated with SUs and determined the rs7903146 diabetes risk genotype. We used a logistic regression with secondary SU failure defined as the addition of insulin after at least 6 months of SU therapy and corresponding A1C measurement of ≥7.0%.
Results: In univariate regression analyses, *TCF7L2* genotype and diabetes duration were the main predictors of SU treatment failure. The rs7903146 T-allele was significantly more frequent in the group of patients additionally treated with insulin (40%) than in the control group treated only with SUs (28 %) [*P*=0.03; odds ratio: 1.73 (1.06-2.84) in an additive mode of inheritance]. The genotype effect was independent of diabetes duration.
Conclusion: Our data suggest that patients with diabetes risk alleles in *TCF7L2* have an altered hypoglycaemic response to SUs resulting in early secondary failure, thus supporting previously reported findings and indicating the potential of pharmacogenomics in the therapy of T2D.

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The role of genetic variation in the sodium-glucose cotransporter 2 gene (*SGLT2*) in the pathophysiology of type 2 diabetes
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Background and aims: The sodium-glucose cotransporter 2 (SGLT2) is the major cotransporter involved in glucose reabsorption in the kidney. Mutations in the *SGLT2* gene cause renal glucosuria and are associated with reduced circulating glucose levels. Treatment with SGLT2-inhibitors results in decreased fasting glucose levels, reduction of HbA1c and lower BMI. We therefore investigated the effects of common genetic variation in *SGLT2* on glucose traits and BMI in non-diabetic subjects as well as the association with type 2 diabetes (T2D).
Materials and methods: Four HapMap tagging single nucleotide polymorphisms (SNPs) (www.hapmap.org) were genotyped (TaqMan, Applied Biosystems, Inc.) for subsequent association studies on BMI, T2D and related metabolic traits in 1046 Sorbs from Germany who had undergone a detailed phenotyping. The SNPs were representative of their linkage disequilibrium groups and were selected according to r^2>0.8 and minor allele frequency >0.01. An independent cohort from Berlin, Germany (N=2046, including 359 subjects with T2D) was taken for replication.
Results: In a case control study including 106 patients with T2D and 786 controls with normal glucose tolerance, none of the SNPs showed association with T2D. However, rs9934336 was nominally associated with 30 min plasma glucose, 2 hr insulin concentrations and incremental $AUC120_{glucose}$ during oral glucose tolerance test in 892 non-diabetic subjects (P<0.05 in additive model adjusted for age, sex and BMI). Carriers of the rs9934336 G-allele had higher 30 min plasma glucose and 2 hr insulin concentrations. The SNP showed no association with T2D in the Berlin cohort, but was, however, nominally associated with 60 min plasma glucose (adjusted P<0.05) and showed consistent effect on $AUC120_{glucose}$ in a subgroup of subjects with impaired fasting glucose and impaired glucose tolerance (N=485).

Conclusion: In conclusion, our data suggest a role of *SGLT2* genetic variation in the regulation of insulin and glucose levels in non-diabetic individuals. *SGLT2* polymorphisms might therefore be potential candidates in pharmacogenomic studies investigating the interaction between these genetic variants and the efficacy of antidiabetic treatment based on inhibition of SGLT2.

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ENPP1 expression and metformin efficacy in type 2 diabetes

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Background: ENPP1 is an inhibitor of insulin signalling whose overexpression plays a role on insulin-resistance. Also the *ENPP1* K121Q polymorphism has been associated with insulin resistance, with the Q121 variant being a gain of function substitution which increases the protein inhibitory activity on insulin signalling. Metformin (Met) is an insulin-sensitizer, established as "first choice" oral hypoglycemic agent (OHA) in type 2 diabetes (T2D). In the Diabetes Prevention Program (DPP) study, the efficacy of Met in preventing future T2D was significantly greater in Q121 carriers (individuals carrying KQ or QQ genotypes) as compared to those carrying the KK genotype, thus suggesting that higher ENPP1 inhibitory activity predicts higher metformin efficacy.
Aims: to investigate i) whether ENPP1 expression predicts the efficacy of 3-month metformin monotherapy on fasting glucose (FG) in patients with T2D; ii) whether Met modulates ENPP1 expression in peripheral blood mononuclear cells (PBMC).
Methods: 55 patients (31 M/24 F; age: 40-70 yrs; disease duration: 2-25 yrs; HbA_{1c}: 6.5-9%; no need of insulin therapy) were recruited. Contraindications to Met treatment were considered as exclusion criteria. Previous OHA were discontinued for 5 days and then Met (2550 mg/daily) was given. Body mass index (BMI), HbA_{1c}, glucose, insulin, insulin-resistance HOMA index, triglycerides and HDL-cholesterol were measured at baseline and 12 weeks after Met treatment. ENPP1 expression levels were measured by quantitative RT-PCR in PBMC before and after treatment.
Results: ENPP1 baseline expression was significantly and directly correlated with Met efficacy as indicated by change in FG after treatment (i.e. baseline FG minus 3-month FG) (adjusted R2=0.09, p=0.015). Of all other measured variables, only baseline FG was able to predict Met efficacy (adjusted R2=0.33, p<0.0001). Of note, ENPP1 maintained a significant prediction ability also when adjusted for baseline FG (p=0.045) as well as when also sex, BMI and duration of T2D were added into the model (p=0.04). Average ENPP1 expression levels didn't change after Met treatment (6.94±6.14 arbitrary units and 7.66±5.78, before and after, respectively; p=0.173).
Conclusion: Our data indicate that Met efficacy is higher in individuals with higher ENPP1 expression and then, presumably characterized by higher ENPP1 inhibitory effect on insulin signalling. These data are very much along the same line of those from the DPP study showing increased metformin efficacy in carriers of *ENPP1* Q121 variant. A better understanding of these phenomena could help setting up strategies aimed at predicting Met efficacy.
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Cyp2c8 variant reduce the therapeutic response to thiazolidinediones - a godarts study

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Background and aims: The reason for highly variable glycaemic response to thiazolidinediones (TZDs) is poorly understood. TZDs are mainly metabolized by the cytochrome p450 2C8 enzyme encoded by CYP2C8. Two common CYP2C8 variants *3 and *4 are associated with greater clearance and lower plasma concentrations of TZDs. We hypothesised that patients carrying these variants would have reduced glycaemic response to thiazolidinediones.
Materials and methods: Linear and logistic regressions were used to model HbA_{1c} reduction and achieving a treatment target of HbA_{1c} <7% in 374 patients from the GoDARTS cohort in Tayside, Scotland. Parameters included are age, gender, BMI, baseline HbA_{1c}, adherence and CYP2C8 genotype.
Results: Compared to the wild-type carriers, model adjusted HbA_{1c} reduction was 0.8% lower in those patients who carry two functional variant alleles (p=0.001). These patients were also 3.8 times more likely to fail achieving treatment target (p=0.06).
Conclusion: In keeping with the pharmacokinetic role of this gene, our data suggest CYP2C8 variants have a marked impact on glycaemic response.
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Resistance to exercise-induced changes in the global DNA methylation pattern of skeletal muscle in individuals with a family history of type 2 diabetes

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Background and aims: First degree relatives of individuals with type 2 diabetes (T2D) have an increased risk of developing the disease. This is conferred by genetic and shared environmental factors, not least physical inactivity, as physical activity is known to improve glucose homeostasis. However, it is unknown if epigenetic changes contribute to the increased risk of T2D. Whether physical exercise can affect methylation of genes of importance for the abnormal glucose metabolism characteristic of individuals with a genetic predisposition to T2D is not known. This study examines the global changes in DNA methylation in skeletal muscle in humans with or without a family history of T2D before and after a six-month exercise intervention.
Materials and methods: 16 men with (FH+) and 13 men without (FH-) a first-degree family history of T2D, matched for age, BMI and VO2max, participated in a supervised six-month exercise intervention study. Biopsies from the vastus lateralis muscle were obtained before and after the exercise intervention. DNA was isolated and MeDIP-Chip was performed e.g. 1 µg DNA was immunoprepitated with a monoclonal antibody against methylated cytosine and hybridized to the NimbleGen 2.1 DeLuxe Array containing 2.1 million probes covering a 10 kB region of all genes, with 7.5 kB upstream and 2.5 kB downstream of the transcription start site in addition to all known CpG islands.
Results: Before exercise, 1891 genes displayed lower methylation and 1237 genes showed higher methylation in skeletal muscle from FH+ vs FH- individuals. Exercise increased methylation of 1402 and decreased methylation of 2136 genes in the whole chohort. Notably, exercise changed methylation of fewer genes in FH+ than in FH- subjects (1085 vs 2355 genes increased, and 2035 vs 3281 decreased methylation) with little overlap between the top 100 genes of the two groups.
Conclusion: Human skeletal muscle of individuals with a family history of T2D is partially resistant to epigenetic changes induced by exercise in individuals with no T2D heredity.
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Does macronutrient intake or physical activity level interact with genetic risk for increased fasting glucose?

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Background and aims: The prevalence of type 2 diabetes (T2D) is drastically increasing around the globe and is believed to be linked to the adoption of a western lifestyle mainly in terms of dietary habits and physical inactivity. Genome-wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNPs) associated with fasting glucose (fGLU) levels and T2D. The aim of this study was to investigate if a combined effect of 15 SNPs previously shown to associate with fGLU levels in GWAS interacts with dietary intakes or physical activity level on fasting glucose levels in the population based Malmö Diet and Cancer Study- Cardiovascular cohort (MDC-CV).
Materials and methods: The 15 SNPs identified in or near *G6PC2, MTNR1B, GCK, DGKB-TMEM195, GCKR, ADCY5, MADD, CRY2, ADRA2A, PROX1, SLC2A2, GLIS3, SLC30A8, FAM148B,* and *TCF7L2* were genotyped by taqman in MDC-CV. After excluding all patients with diabetes, individuals without diet-data and those with incomplete genotype information we included 4615 individuals in the study (41% males, age 57±6 years, BMI 26±4 kg/m^2, fGLU 5.7±0.8 mmol/l). A fGLU genetic risk score (fGLU-GRS) was

created summing the number of fGLU increasing alleles of the 15 SNPs. Assuming additive model and adjusting for age and sex we analysed association between fGLU-GRS and fGLU. Association between GRS and fGLU was evaluated in strata of gender-specific tertiles according to percentage of energy from macronutrients as well as physical activity score. Interaction between dietary factors or physical activity and fGLU-GRS was assessed by introducing a multiplicative factor with continuous variables adjusting for age, sex, season and total energy intake.
Results: The fGLU-GRS was strongly associated with fGLU (p=5.8e-15) with a mean effect size of 0.04 mmol/l per each glucose increasing allele. Similar associations were found in males (p=1.7e-6) and females (p=2.4e-10). Individuals in the highest fGLU-GRS quintile had 0.26 mmol/l higher fGLU compared to those in the lowest quintile. The effect sizes of each fGLU increasing alleles were 0.04, 0.06 and 0.02 mmol/l within low, medium and high carbohydrate intake tertiles (p=0.11, p=0.09 and p=0.71 for interaction in all, males and females, respectively); 0.05, 0.04 and 0.04 mmol/l within low, medium and high fiber intake tertiles (p=0.27, p=0.50 and p=0.43) and 0.02, 0.05 and 0.04 mmol/l in low, medium and high fat intake tertiles (p= 0.23, p=0.16 and p=0.89). The mean effect size of each fGLU increasing allele was 0.4 mmol/l in all physical activity tertiles (p=0.84, p=0.50 and p=0.65 for interaction in all, males and females, respectively).
Conclusion: GRS of 15 fGLU SNPs associated strongly with fGLU in a population based Swedish sample. Our study did not reveal significant interactions between such genetic susceptibility and macronutrient intakes or physical activity level. We believe that larger sample sizes and taking into account the quality of dietary carbohydrates and fat need to be taken into account in future studies to exclude such interactions.
Supported by: Swedish Medical Research Council, Swedish Diabetes Foundation and LUDC

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Body mass index is a potential modifier of the influence on beta cell function exerted by SLC30A8 and KCNJ11 diabetes risk variants in patients with newly diagnosed type 2 diabetes
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Background and aims: The zinc transporter, SLC30A8, and the potassium channel, KCNJ11, are both expressed in the beta cell and are involved in insulin transport and secretion. The rs13266634 G allele in SLC30A8 and the rs5219 A allele in KCNJ11 are associated with type 2 diabetes (T2D) risk and with reduced beta cell function (BF) in non-diabetic subjects. We evaluated the role of these two non-synonymous polymorphisms in determining clinical and pathophysiological traits in patients with newly diagnosed type 2 diabetes.
Materials and methods: 456 GAD-negative and drug-naive patients (age 57.8±10.3 years, BMI 29.9±5.1 kg/m^2, HbA1c 7.0±1.4 %) with newly diagnosed type 2 diabetes underwent standard clinical characterization. BF and insulin sensitivity (SI) were assessed by mathematical modeling of glucose/C-peptide curves during a 240' frequently sampled OGTT and by euglycemic insulin clamp, respectively. The beta-cell responses to the rate of increase of glucose (G) (derivative or dynamic control: DC; median[IQ range]: 421[0.6-907] [pmol · m^{-2} BSA]/[mM · min^{-1}]) and to G concentration (proportional or static control, PC, presented as the insulin secretion rate at G concentrations of 5.5, 8.0, 11.0, 15.0 and 20.0 mM, respectively; 160±69, 223±122, 359±218, 572±376, 843±591 pmol · min^{-1} · m^{-2} BSA) are herein reported as measures of BF. SI is presented as the M value in the last 60' of the clamp (561[355-796] µmol · min^{-1} · m^{-2} BSA). Rs13266634 in SLC30A8 and rs5219 in KCNJ11 were genotyped in all patients.
Results: In obese patients (n=202 with BMI>=30), but not in the nonobese (p=0.40), the rs13266634 G allele of *SLC30A8* (frequency: 0.74) was associated with reduced PC (-8.5±7.7, -27.3±13.8, -60.3±24.4, -93.9±45.8, -136±74.3 at G concentrations of 5.5, 8.0, 11.0, 15.0 e 20 mM; p<0.04) and with increased levels of glucose at 2 hours during OGTT (+0.80±0.33 mmol/l, p<0.02). In nonobese patients (n=254 with BMI<30), but not in the obese (p=0.64), the rs5219 A allele of KCNJ11 (frequency: 0.39) was associated with reduced DC (-157±58; p<0.01) according to an additive model, and to both reduced PC (-6.9±10.8, -38.7±21.1, -86.8±38.3, -135±63.4, -167±97.2 at G concentrations of 5.5, 8.0, 11.0, 15.0 e 20 mM; p<0.05) and increased fasting plasma G (AA: +0.81±0.33 mmol/l; p<0.02) according to a recessive model.
Conclusion: In patients with newly diagnosed type 2 diabetes the non-synonymic variants of SLC30A8 and KCNJ11 herein investigated are associated with worse BF, with BMI (obesity) apparently playing a modifying role on this relationship. These data, if confirmed, could be useful for diagnostic, therapeutic and clinical purposes.
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The presence of CAD significantly modulates the diabetes risk conferred by the TCF7L2 rs7903146 variant
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Background and aims: Genetic variant rs7903146 in the transcription factor 7-like 2 (TCF7L2) gene has been consistently associated with type 2 diabetes (T2DM) in several studies. It is unknown whether it confers the same amount of diabetes risk in patients with CAD as in patients who do not have CAD. We therefore aimed at investigating whether the presence of CAD modulates the association of TCF7L2 variant rs7903146 with T2DM.
Materials and methods: We therefore performed genotyping of variant rs7903146 in a large cohort of 1650 consecutive Caucasian patients undergoing coronary angiography for the evaluation of established or suspected CAD. At angiography, significant CAD was diagnosed in the presence of significant coronary stenoses with lumen narrowing of ≥50%. The association between rs7903146 and T2DM was evaluated in an additive genetic model.
Results: Variant rs7903146 was significantly associated with the presence of T2DM in the total study cohort (adjusted odds ratio (OR)=1.38 [1.15-1.65]; p<0.001). Also, diabetes duration significantly (p=0.024) increased from the CC over the CT to the TT genotype (7.7±8.6, 8.1±7.3 and 9.2±6.8 years). When patients with CAD (n=950) were analyzed separately from those without significant CAD, the association between variant rs7903146 and T2DM was strongly significant in patients with significant CAD (adjusted OR=1.59 [1.26-2.00]; p<0.001), but not in subjects who did not have significant CAD (OR=1.04 [0.77-1.40]; p=0.807). Variant rs7903146 was also significantly associated with diabetes duration in individuals with CAD (7.9±8.8 8.9±7.4 and 9.3±6.8 years for the CC, CT, and TT genotype, respectively, p=0.018), but not in patients without significant CAD (p=0.718). An interaction term CAD x rs7903146 was significant (p=0.018), indicating a significantly stronger impact of the polymorphism on T2DM risk in patients with significant CAD than in subjects without significant CAD.
Conclusion: We conclude that the presence of CAD significantly modulates the diabetes risk conferred by the TCF7L2 rs7903146 variant.

PS 7 Genetics of diabetic complications, related metabolic traits

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Allelic variations in the catalase gene are associated with development and progression of diabetic nephropathy in subjects with type 1 diabetes

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Background and aims: Oxidative stress is involved in the pathophysiology of diabetic nephropathy (DN). The antioxidant enzyme Catalase plays a major role in the detoxification of reactive oxygen species and thus could have a protective role against DN. In this study, we tested the impact of allelic variation in the Catalase gene (CAT) on the development and progression of DN in subjects with Type 1 Diabetes Mellitus (T1DM).
Materials and methods: Twelve SNPs (table 1), giving information on ~90% of the allelic variation of the haplotypic block containing CAT gene were analyzed in 1463 subjects from three independent T1DM cohorts: the SURGENE prospective study (follow-up of 10 ± 3 years, mean ± SD), GENEDIAB and GENESIS studies. Genotypes were determined by an Assay by Design kit from Applied Biosystems. Genotype associations with DN were assessed by logistic regression analyses. Associations with DN severity were assessed by ordinal logistic regression analyses, with stages of DN coded as ordinal polytomic dependent variables: absence (1), microalbuminuria (2), macroalbuminuria (3), reduced renal function (4) and end stage renal failure (5).
Results: In the SURGENE cohort, the rs7947841 variant was associated with DN both at baseline (Odds Ratio 8.60, 95% C.I. 1.83 - 40.32, p=0.005) and at follow-(Odds Ratio 4.34, 95% C.I. 1.29 - 14.78, p=0.01). The variant was also associated with the severity of DN, both at baseline (p=0.005) and at follow-up (p=0.001). In GENEDIAB cohort, five SNPs were associated with DN (Table 1). These SNPs were also associated with the severity of DN severity (p=0.009, p=0.06, p=0.02, p=0.006 and p=0.006, respectively) and with microalbuminuria (p=0.03, p=0.05, p=0.04, p=0.01 and p=0.003, respectively). We have also observed associations of these variants with arterial hypertension.
Conclusion: We have observed associations of CAT allelic variations with diabetic nephropathy, its severity and with intermediate phenotypes in subjects with T1DM. We are currently performing haplotypic studies in the 3 cohorts and analysis of 6 years follow-up data of GENESIS and GENEDIAB cohorts.

Table 1: GENEDIAB study: Association of CAT polymorphisms with diabetic nephropathy

SNP	Diabetic nephropathy at baseline	
	Odds ratio (95% IC)	p*
rs2266630	1.17 (0.41 - 3.66)	0.76
rs1001179	2.84 (1.24 - 6.79)	0.01
rs11032699	1.23 (0.63 - 2.42)	0.54
rs12272630	0.18 (0.04 - 0.77)	0.02
rs2300182	0.77 (0.33 - 1.84)	0.56
rs11032703	1.22 (0.40 - 3.87)	0.73
rs2300181	0.65 (0.32 - 1.33)	0.24
rs10488736	0.44 (0.22 - 0.88)	0.02
rs2420388	1.00 (0.49 - 2.08)	0.99
rs566979	0.44 (0.23- 0.86)	0.01
rs7947841	1.87 (0.55 - 6.73)	0.32
rs499406	2.14 (1.07 - 4.32)	0.03

*Adjusted for sex, age, duration of diabetes, HbA1c and ACE inhibitors

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Does genetic variability in the fructosamine-3-kinase play a role in the progression of diabetic nephropathy, morbidity and mortality of diabetics?

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Background and aims: Fructosamines are products of non-enzymatic glycation formed in accelerated rate during hyperglycemia. As precursors of advanced glycation end-products (AGEs) fructosamines supposedly contribute to the development of glucotoxic injury. Mechanism of enzymatic deglycation of proteins *in vivo* by fructosamine-3-kinase (FN3K) was described recently. FN3K is a ubiquitous intracellular enzyme that phosphorylates fructosamines resulting in unstable fructosamine-3-phosphate, which subsequently spontaneously decomposes to inorganic phosphate, 3-deoxyglucosone and the unmodified amine. Recently, the -385A/G (rs3859206) and 900C/G (rs1056534) single nucleotide polymorphisms (SNPs) in the FN3K gene were found to have potential functional impact - association with FN3K enzyme activity in erythrocytes (genotypes -385AA and 900GG associated with lowest enzymatic activity). Fructosamine pathway may therefore represent either potentially protective metabolic process in hyperglycemia since degradation of fructosamines prevents formation of Lys-based AGEs or quite the reverse - harmful process - by formation of 3-deoxyglucosone as a potent mobile Arg-directed glycating agent. The aim was to study relationship between polymorphisms in FN3K gene, progression of diabetic nephropathy (DN) and cardiovascular morbidity and mortality of diabetics.
Materials and methods: Study comprised a total of 420 T1DM or T2DM subject with variable stage of DN (i.e. normoalbuminuria, microalbuminuria, proteinuria or ESRD) prospectively followed for 45 [21 - 63] months (median [IQR]). Following end-points were considered: [1] renal (progression of DN by stage or reaching the ESRD), [2] major cardiovascular event (MCVE: non-fatal myocardial infarction or stroke, limb amputation), [3] cardiovascular mortality (CVM: fatal myocardial infarction, stroke or sudden death) and [4] all-cause mortality (AM). SNPs were genotyped by PCR with subsequent RFLP.
Results: Progression of DN was reached in 16.5% of subjects, MCVE in 15.6%, CVM in 9.9% and ACM in 19.3%. Allele and genotype frequencies did not differ between DN stage groups (chi-square test). Using Kaplan-Meier time-to-event analysis significant effects were ascertained for the carrier state of the -385AA and 900GG genotype combinations and progression of DN, MCVE and CVM (all P<0.05, log-rank test). In all cases, group defined by the presence of at least one "low-activity" allele in both positions (i. e. -385AA or AG combined with 900GG or GC) were associated with significantly longer median of time to DN progression, MCVE and CVM.
Conclusion: Interindividual variability in FN3K enzyme activity represents potentially significant genetic risk factor for the progression of DN and cardiovascular morbidity and mortality of diabetics. Based on our results, we can't identify high FN3K deglycating activity as a protective factor, on the contrary higher rate of 3-deoxyglucosone formation may counterbalance putative protection by providing substrate for Arg-directed glycation and AGE formation.
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***PPAR-γ2* P12A polymorphism and albuminuria in patients with type 2 diabetes**

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Background and aims: Insulin resistance (IR) is believed to be pathogenic for albuminuria in patients with T2D. The *PPAR-γ2* P12A polymorphism has been consistently associated with IR and T2D with the A12 variant playing a protective role. The association of this variant with a reduced risk of albu-

minuria in T2D has been controversial. The aim of this study was to investigate the relationship between PPAR-γ2 P12A polymorphism and albuminuria in patients with T2D.
Materials and methods: To get deeper insights about this issue, we first tested the association between the A12 variant and albuminuria in two new case-control studies in patients with T2D from Italy, and then performed a meta-analysis of all studies available to date. The first study comprised 261 cases (162 male/99 female; age, 63.2±10.0 yrs) and 580 controls (264 male/316 female; age, 61.6±9.5 yrs) recruited at Scientific Institute CSS in San Giovanni Rotondo. The second study comprised 254 cases (131male/123 female; age, 63.9±12.2 yrs) and 369 controls (170 male/199 female; age, 62.2±12 yrs) recruited at University Hospital of Foggia. In these 2 studies, albuminuria was defined if ACR was ≥2.5 in men and 3.5 mg/mmol in women. Pro12Ala polymorphism was genotyped by TaqMan-based assay in genomic DNA. The 8 studies we meta-analyzed comprised 2144 cases and 3706 controls. In four studies albuminuria was determined by albumin excretion rate (AER) and in four by albumin concentration in a single spot (i.e. urine albumin/creatinine ratio in 3 studies and urinary albumin concentration in one study).
Results: The overall OR (95% CI) for association between A12 and albuminuria was 0.67 (0.49-0.91), with values of individual studies ranging from 0.29 to 1.13. A significant heterogeneity of the genetic effect was observed (Cochran's *Q* test p=0.017). Approximately one third of it (36.4%) was explained by the different method of urine collection and albuminuria definition utilized across the studies. Most of the protective effect of the A12 variant was observed in the 4 studies using AER rather than in those using albumin concentration in a single spot (OR, 95% CI: 0.52, 0.34-0.79, p=0.0024 and 0.84, 0.58-1.21, p=0.35, respectively).
Conclusion: The present meta-analysis shows that the PPARγ2 Ala12 variant is significantly associated with a reduced risk of albuminuria among patients with T2D. This association is particularly evident among studies where the ascertainment of case-control status was obtained by measurement of albumin excretion rate.

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INPPL1 gene is associated with the metabolic syndrome but not with diabetic nephropathy in patients with type 1 diabetes

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Background and aims: The metabolic syndrome has been shown to be a frequent phenomenon in patients with type 1 diabetes and to associate with diabetic nephropathy. *INPPL1* gene encodes lipid phosphatase SHIP2, a negative regulator of PI3-kinase mediated insulin signaling. Polymorphisms in *INPPL1* gene have been found to associate with components of the metabolic syndrome in British and Japanese cohorts. The aim of this study was to investigate if single nucleotide polymorphisms (SNPs) in *INPPL1* are associated with the metabolic syndrome or diabetic nephropathy in Finnish patients with type 1 diabetes.
Materials and methods: We selected 2520 patients participating in the FinnDiane study for this cross-sectional study. The metabolic syndrome was defined according to the most recent criteria (joint statement 2009), and patients were divided into controls without the metabolic syndrome (n=1010) and cases with the metabolic syndrome (n=1475), as well as into four groups based upon their albumin excretion rate: normoalbuminuria (n=1256), microalbuminuria (n=442), macroalbuminuria (n=553) and end stage renal disease (n=266). Nine SNPs were selected for genotyping from the HapMap database (CEPH, r^2>0.8) for *INPPL1* gene plus/minus 20 kbs. Genotyping was performed with ABI Prism 7900 Sequence Detection System based on TaqMan chemistry. The associations between the SNPs and outcome variables were analysed with the Chi-squared test.
Results: Two *INPPL1* SNPs, rs2276047 (in an intron) and rs2276048 (silent mutation), were found to associate with the metabolic syndrome in males, with p-values 0.018 and 0.001, respectively. When both genders were included, the association was not significant. No association between the genotyped SNPs and various degrees of nephropathy was observed.
Conclusion: *INPPL1* gene variants may contribute to susceptibility to the metabolic syndrome, however, not to diabetic nephropathy in patients with type 1 diabetes.
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CNDP1 gene polymorphism predicts cardiovascular mortality in female patients with type 2 diabetes (ZODIAC 22)

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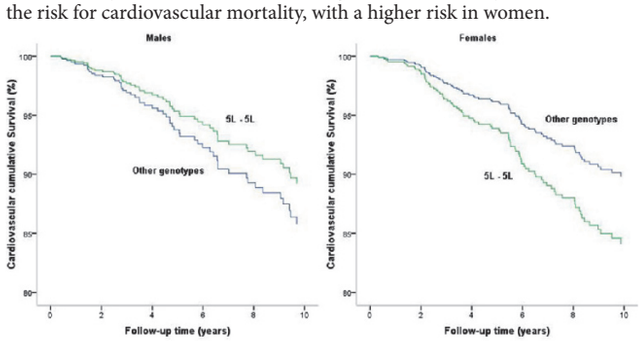
Background and aims: Carnosine has been shown to be protective against oxidative stress and endothelial damage. Homozygosity for 5-leucine repeat (5L-5L) in the carnosinase gene (CNDP1) has recently been found to be associated with diabetic nephropathy in cross-sectional studies, mainly in women with type 2 diabetes. Longitudinal data on survival are, however, not yet available. We prospectively investigated whether allelic variation in CNDP1 predicts (cardiovascular) mortality in patients with type 2 diabetes and to what extent this is modified by sex.
Materials and methods: This study is part of the ZODIAC study, a prospective observational study in primary care patients with type 2 diabetes. Leucine repeats in CNDP1 were assessed by fluorescent DNA analysis. In 2009, data on mortality were collected. A Cox proportional hazard model was used to compare 5L-5L with other allelic variations, after adjustment for potential confounders, including age, body mass index, duration of diabetes, macrovascular complications, systolic blood pressure, HbA_{1c}, total cholesterol to HDL-cholesterol ratio, plasma creatinine, and urine albumin excretion. Furthermore, CNDP1 and sex were entered as an interaction term.
Results: 872 patients [age 68.1 ± 11.2 years, 366 (42%) males] were included. At baseline, median diabetes duration was 5 (interquartile range (IQ) 2-11) years, mean HbA_{1c} 56 ± 12 mmol/mol, plasma creatinine 91 (IQ 82-104) μmol/L, and urinary albumin creatinine ratio 1.94 (IQ 0.95-6.18) mg/mmol. 5L-5L was found in 331 patients (38%; this was for men 39% and for women 37%). After 9.5 (IQ 5.9-10.3) years of follow-up, 136 (41%) patients with 5L-5L had died, with 55 deaths (17%) attributable to cardiovascular causes. In patients with other allelic variants, these numbers were 205 (38%) and 81 (15%), respectively. Adjusted hazards ratios (HR) for all-cause mortality and cardiovascular mortality in 5L-5L versus other allelic variants were 1.08 (95%CI 0.87-1.35) and 1.12 (95%CI 0.79-1.60), respectively. There was a significant interaction between allelic variation in CNDP1 and sex for prediction of cardiovascular mortality (*P*=0.02), but not for all-cause mortality (*P*=0.69). After stratification for sex, adjusted HRs of 5L-5L for cardiovascular mortality were 0.74 (95%CI 0.42-1.32, *P*=0.31) in males and 1.61 (95%CI 1.02-2.55, *P*=0.04) in females.
Conclusion: In this prospective study on allelic variation in CNDP1 in patients with type 2 diabetes, 5L-5L was gender-dependently associated with the risk for cardiovascular mortality, with a higher risk in women.



Adjusted cardiovascular survival curves in male and female patients with type 2 diabetes according to their CNDP1 genotype

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Two common variants on 9p21 affect mortality risk in type 2 diabetes patients (ZODIAC-15)

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Background and aims: Recent genome wide association (GWA) studies identified two single nucleotide polymorphisms (SNP), rs10811661 and rs10757278 in the same region on the 9p21 chromosome to be consistently and independently associated with the risk of developing type 2 diabetes (T2DM) and cardiovascular disease, respectively.
We examined the SNPs in relation to the risk of total and cardiovascular mortality in a population based cohort of T2DM patients.
Materials and methods: The ZODIAC study is a prospective cohort study of T2DM patients treated in primary care in the Netherlands. Rs10811661 and rs10757278 were genotyped in 914 subjects from the ZODIAC study and 920 healthy Dutch controls. Associations of the SNPs with mortality were assessed by use of Cox proportional hazard analyses.
Results: After a median follow-up of 9.5 years 358 out of 914 patients had died. The adjusted Hazard Ratio's (HR) for total mortality for patients homozygote and heterozygote for the wild type allele of rs10811661 was 1.34 (95% CI 1.06-1.69, p=0.01) compared to individuals homozygous for the risk allele. For rs10757278 total mortality was lower among patients heterozygous and homozygous for the wild-type allele than in homozygous carriers for the risk allele (HR 0.64 (95%CI 0.43-0.95), p=0.03, and HR 0.73 (95%CI 0.48-1.11), p=0.14, respectively). This effect was more pronounced in the lower tertile of HbA1c: the adjusted HR for patients heterozygous and homozygous for wild-type allele of rs10757278 was 0.48 (95%CI 0.28-0.83, p=0.01) and 0.48 (95%CI 0.27-0.85, p=0.01), respectively, compared with the patients homozygous for the risk allele.
Conclusion: This prospective study shows a significant association between two common SNPs on 9p21 and mortality in type 2 diabetes patients.

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Genome wide association analysis for free fatty acid levels in DGI

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Background and aims: Recent genome-wide association studies (GWAS) have identified a number of common single nucleotide polymorphisms (SNPs) that contribute to multifactorial human phenotypes. Free fatty acids (FFAs) serve as physiologically important energy substrates and their release from adipose tissue is regulated by the energy demands of the body. FFAs are increased in obese patients and T2D and cause insulin resistance in all major insulin target organs. Although FFA levels have a heritability of 50-60%, very little is known about contributing genetic factors. The aim of this study is to identify genetic factors regulating FFA levels using a GWAS approach.
Materials and methods: In total 1094 non-diabetic unrelated individuals (49% males, age 59±11 years, BMI 27±4 kg/m^2) from the Diabetes Genetics Initiative (DGI) GWAS of 500K were included in the analyses. FFA-levels were determined using Half-micro and Micro 96-plate methods with two different kits NEFA HR (2) and NEFA C (Waco), after an overnight fast (FFA0) and after an OGTT (FFA120) and a dFFA was calculated reflecting insulin suppression (FFA0-FFA120). FFA-levels of the 1094 individuals were FFA0 655±235 μmol/l, FFA120 221±114 μmol/l and dFFA 432±221. Linear regression analyses were performed in plink with age, sex, fasting insulin, BMI and region or age, sex and region as covariates. Replication analyses are performed among 2845 participants of the Botnia Prevalence, Prediction and Prevention of Diabetes study (Botnia-PPP: 47% males, age 48±15 years, BMI 27±4 kg/m^2, FFA0 593±246 μmol/l, FFA120 159±80 μmol/l and dFFA 431±229).
Results: In DGI, the lowest p-values for FFA0, FFA120 and dFFA were found in or near *TUB*, *UBE2NL/SPANXN2* and *c4orf32/TIFA* loci on chromosomes 11, 23 and 4 (p= 1x10^{-6}, p=1x10^{-5} and p=4x10^{-6}, respectively). In total 24, 15 and 29 SNPs provided p-values of less than 1x10^{-4} for FFA0, FFA120 and dFFA, respectively. 14 loci associated with both FFA120 and dFFA with p<1x10^{-4}. All SNPs with p<1x10^{-4} in DGI were chosen for replication in Botnia-PPP. Similar to DGI, the *TUB* SNP associated with significantly lower dFFA in PPP (p=0.0005) while association with FFA0 did not reach statistical significance (p=0.065). These results were similar with and without adjustment for fasting insulin and BMI.
Conclusion: A GWAS for FFA identified 64 loci with p<1x10^{-4} and replication genotyping of these loci is ongoing. Our results so far provide evidence for involvement of *TUB* gene in regulation of FFA levels in man. *TUB* codes for the human homolog of a gene that causes maturity onset obesity and insulin resistance in *tubby* mice.

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Interpretation of genome wide association data for lipoprotein traits using systems biology approach

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Background and aims: Genome wide association studies (GWAS) with large meta-analyses have identified a number of new loci contributing to multifactorial diseases. Hereby we assumed that by analysis of interaction networks of GWAS genes with SNPs providing P <5x10^{-8} (seed genes) in meta-analysis data of Global Lipids Genetics Consortium (GLGC) including > 100,000 individuals it could be possible to reveal novel candidate genes and pinpoint important pathways for lipid and lipoprotein traits. By integrating GLGC meta-analysis data with Molecular networks (expression, transcriptional, proteomic and metabolic interaction networks) as well as with phenotypic disease network (comorbidity network), we wish to gain new biological insights for better understanding of lipoprotein metabolism.
Materials and methods: GLGC meta-analysis data of ~ 2.6 million genotyped or imputed SNPs for four lipid/lipoprotein traits i.e. total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) was analyzed. After correcting for linkage disequilibrium, SNPs were assigned to genes on the basis of being located within 20kb region of first and last exon. We defined a seed genes data set (association p-value <5x10^{-8}) of 112 genes for HDL-C, 117 for LDL-C, 158 for TC and 115 for TG. Interaction information gleaned from the first order neighbours and second order neighbours were used in further analysis. We then approached to identify additional candidate genes by studying clusters of seed genes using Molecular Triangulation (MT) algorithm. In the next step we only selected genes that were co-expressed with the seed genes using 79 human tissues expression data. Further, the GLGC meta-analysis p-values of seed genes were superimposed on the interaction networks to identify the sub-networks and additional 100 random control networks were generated. For weighing the identified sub-networks for comorbidity we utilized the large scale US Medicare database of comorbidity patterns of 13 millions patients.
Results: By MT algorithm we identified 223, 172, 140 and 243 additional candidate genes for HDL-C, LDL-C, TG and TC with significance of real scores of P=5.4x10^{-5}, P=4.7x10^{-5}, P=4.4x10^{-5} and P=6.5x10^{-5}, respectively. Receiver operating characteristic (ROC) curve showed accuracy of 93% and sensitivity of 64% for MT algorithm with the seed genes. After priorization of those additional candidate genes that co-expressed with seed genes and were present in the sub-networks having highest comorbidity values we were left with 39, 19, 27 and 20 genes for HDL-C, LDL-C, TG and TC. Of these genes, lowest p-values in GLGC meta-analysis data were for HDL-C in *INSR* (P=2x10^{-5}) and *ASCC2* (P=0.0005), for LDL-C in *SH3GL2* (P=0.0001) and *NDUFA4L2* (P=0.0003), for TG in *GCK* (P=3.7x10^{-5}) and *RAF1* (P=8.6x10^{-5}) and for TC in *SH3GL2* (P=0.0002) and *GOLM1* (P=0.004).
Conclusion: Combining large GWAS meta-analysis data with systems biology approaches and comorbidity data identified new candidate genes for lipoprotein traits and addresses the importance of network based genetic analyses in the future.
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Association of FTO gene variation with fat oxidation in women with polycystic ovary syndrome

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Background and aims: Polycystic ovary syndrome (PCOS) is heterogeneous disorder, where insulin resistance might be involved in the development of endocrine and metabolic abnormalities. It was recently shown that the *FTO* gene modifies weight, fat mass and insulin sensitivity in women with polycystic ovary syndrome, where its role might be larger than in other phenotypes. The aim of the present study was to estimate the effect of *FTO* variation on glucose and lipid oxidation in PCOS women.
Materials and methods: The study group consisted of 68 women with PCOS and 25 healthy, normally menstruating women. Clinical examination, anthropometric measurements, euglycemic hyperinsulinemic clamp and the measurements of serum sex hormones were performed. Glucose and lipid oxidation was evaluated with indirect calorimetry in the baseline state and during the last 30 minutes of the clamp. The *FTO* rs9939609 polymorphism was genotyped using the restriction fragment length polymorphism method.
Results: There was no difference in glucose and lipid oxidation between PCOS and control women. TT homozygotes had higher baseline fat oxidation in comparison to the carriers of A allele (p=0.019) in the entire study population. We found similar differences when PCOS women were analyzed separately (p=0.018). We did not observe the effect of *FTO* gene variation on insulin-stimulated lipid oxidation and either baseline or insulin-stimulated glucose oxidation.
Conclusion: Our data show that *FTO* gene variation might influence baseline lipid oxidation in PCOS patients. This might be one of potential mechanism explaining the impact of the *FTO* gene on body weight.
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Impact of the I148M mutation in PNPLA3 (adiponutrin) on weight loss-induced decrease in liver fat

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Background and aims: The rs738409 C>G single nucleotide polymorphism (SNP) in the patatin-like phospholipase domain-containing 3 (PNPLA3; adiponutrin) gene leads to a missense mutation (I148M) in PNPLA3. Carriers of the GG genotype (prevalence ~5%) have a marked 60% increase in liver fat. *In vitro*, adiponutrin has been associated with both transacylase and lipase activities. We explored whether the I148M variant in the adiponutrin gene influences weight loss -induced decrease in liver fat in humans.
Materials and methods: We recruited 17 subjects of whom 8 had the GG genotype and 9 the CC genotype. We matched the groups with respect to age (48 ± 4 vs. 53 ± 4 yrs, GG vs. CC, NS), BMI (29.2 ± 2.1 vs. 31.5 ± 1.9 kg/m^2, NS) and liver fat (10.2 ± 1.8 vs. 13.2 ± 1.8 %, NS). The subjects were placed on a hypocaloric (1000 kcal deficit/ day) low-carbohydrate (<20 g/ day) diet for six days. Liver fat was measured by proton magnetic resonance spectroscopy before and after the intervention. Whole-body insulin sensitivity was measured by the euglycemic insulin (0.4 mU * kg^{-1} * min^{-1}) clamp technique.
Results: Weight loss was similar in both groups (-3.7 ± 3.5 vs. -3.3 ± 0.3 kg, GG vs. CC, NS). Liver fat decreased by 45 % in the GG and by 20 % in the CC group (p=0.046). Whole-body insulin sensitivity increased significantly in the GG group from 0.90 ± 0.18 to 1.33 ± 0.25 mg * kg^{-1} * min^{-1} (p=0.04) and remained unchanged in the CC group (0.62 ± 0.10 vs. 0.68 ± 0.11 mg * kg^{-1} * min^{-1}, p=0.58). No significant differences between changes in fasting concentrations of triglycerides, glucose or insulin were observed.
Conclusion: These data suggest that the I148M mutation facilitates weight loss -induced mobilization of intrahepatocellular triglyceride *in vivo* in humans.

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Three year-follow-up incidence of cardio-metabolic alterations in a metabolically normal population

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Background and aims: It has been claimed that obese subjects with normal metabolic profile are not at increased risk for cardio-metabolic diseases, as compared to normal body weight. The aim was to compare the 3-year evolution of cardio-metabolic parameters in normal body weight (NBW) and obese (OB) healthy subjects, free from MS and with a normal glucose tolerance at baseline.
Materials and methods: We investigated a sub-group of the RISC study (Relationship between Insulin Sensitivity and Cardiovascular Disease) participants (NBW=288 and OB=141) free from metabolic syndrome (MS, according to IDF criteria) and with a normal glucose tolerance at baseline. In these subjects, fasting insulin was measured and insulin sensitivity was assessed by euglycaemic hyperinsulinaemic clamp. Based on quartiles established in NBW, both NBW and OB subjects were classified as normo-insulinaemic or hyper-insulinemic and insulin sensitive or insulin resistant. Metabolic normality was defined as the presence of normal levels of both fasting insulin and insulin sensitivity. Three years later, the cardio-metabolic parameters suggested by the IDF to define MS as well as glucose tolerance were re-measured.
Results: At 3 years, the incidence of MS was 6.6% in NBW and 21.3% in OB (p<0.0001), the incidence of impaired fasting glucose was 7.6% vs 20.6% (p<0.0001) and of impaired glucose tolerance 3.8% vs 10.6% (p=0.005), in NBW and in OB respectively. Hypertension occurred in 6.3% of NBW vs 14.9% in OB (p=0.003). In the overall population, both BMI at baseline and BMI modifications along the 3-year period were predictors for MS (OR=1.12, p=0.04 and OR=1.44, p=0.01, respectively), whereas BMI at baseline was predictor of impaired fasting glucose (OR=1.20, p=0.001). BMI evolution predicted impaired glucose tolerance (OR=1.44, p=0.02) and hypertension (OR=1.47, p=0.04).
Conclusion: Even when metabolically normal, OB subjects show an increased risk for MS, pre-diabetes and hypertension. Therefore, they need a closer surveillance.
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Gestational diabetes is not associated with adiposity measurements in 18 months offspring: the mother child rhea cohort in Crete, Greece

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Background and aims: Studies of developmental origins of health and disease have put focus on the possible role of intrauterine exposure to hyperglycemia in the pathogenesis of obesity and cardiovascular disease in offspring. Although gestational diabetes is a strong risk factor for obesity in the offspring, the age at which this association becomes apparent is unknown. The purpose of this study was to examine the relation of gestational diabetes with measures of adiposity in early childhood.
Materials and methods: The mother-child "Rhea"study in Crete is a prospective cohort examining pregnant women (Greek and immigrants) residents at the prefecture of Heraklion that became pregnant during one year starting in February 2007 and initiated prenatal care before 15 weeks of gestation (mean: 12 weeks). Six hundred and thirty five pregnant women and their children, were included in the analysis. Pregnant women were screened for gestational diabetes (GDM) between 24 and 28 weeks of gestation, and GDM was defined by the criteria proposed by Carpenter and Coustan. Weight, height, abdominal circumference, and thickness of triceps, subscapular, suprailiac, and subscapular were measured in 635 children at 18 months of age (59 offspring of mothers with gestational diabetes). Multivariable linear and logistic regression models were used to estimate the effect of GDM on the risk of adiposity in early childhood after adjusting for offspring sex, age, maternal education, and parity.

Results: Offspring of mothers with gestational diabetes did not differ significantly in BMI (beta coefficient: -0.20, 95% Confidence Intervals: -0.83-0.44), abdominal circumference (beta coefficient: 0.30, 95% Confidence Intervals: -0.65-1.25), or body fat percentage (beta coefficient: -0.27, 95% Confidence Intervals: -1.59-1.05) compared with offspring of non-diabetic mothers after adjustment for offspring sex, age, maternal education, and parity. Similarly, adiposity rates (BMI≥85th percentile-Odds ratio: 0.96; 95% Confidence Intervals:0.44-1.55 , abdominal circumference≥85th percentile-Odds ratio: 1.29; 95% Confidence Intervals: 0.59-2.84, sum of skin folds >85th percentile-Odds ratio: 1.14; 95% Confidence Intervals: 0.20-1.26, and percent body fat >85th percentile-Odds ratio: 0.95; 95% Confidence Intervals: 0.36-2.55) did not differ significantly between the two groups.
Conclusion: The study found no association between gestational diabetes and obesity in early childhood. These findings are consistent with the few other studies with adiposity measurements in early childhood. Further follow up of this cohort will allow determining if gestational diabetes has, an effect on obesity and cardiovascular risk in later childhood.
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Body mass index and fasting glucose levels in a large cohort of patients assigned to age decades between <20 and >80 years - relationship with cardiovascular events and medication

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Background and aims: There is an ongoing debate about the relationship between obesity and morbidity in the elderly and the clinical relevance of overweight in older patients. The main purpose of our study was to investigate whether a higher BMI is associated with an increase in fasting glucose levels and cardiovascular morbidity in all age groups.
Subjects and methods: We performed a retrospective evaluation of clinical data from 5374 patients who visited a medical outdoor center for diagnostic and/or therapeutic interventions in the period from January 1995 to September 2009. Patients were assigned to eight age groups of one decade from <20 years to ≥80 years and results were analyzed with respect to the presence or absence of cardiovascular events and need for medication.
Results: The Body Mass Index (BMI) revealed a hump-shaped pattern, with a peak in the age group 60-69. In all age groups, there was a significant difference in the BMI in patients with and without a cardiovascular event. BMI and the percentage of body fat were higher in all patients with cardiovascular events. The fasting glucose values increased continuously in the patients without events from 85.7±15.9 mg/dL in the age group <20 to 115.6±40.4 mg/dL in patients ≥80 years. In patients with events fasting glucose values increased up to 134.8±61.6 mg/dL in the age group 40-49, and, probably because therapeutical interventions were begun at that point, no further increase could be observed with increasing age. The analysis of patients with and without a need for medication demonstrated that patients with a need for medication revealed higher fasting glucose levels in the age groups between <20 and 60-69 years. Fasting glucose values showed a continuous increase with increasing age with the highest values in the age group 70-79 in patients without medication (112.2±30.4 mg/dL) and in the age group ≥80 in patients with medication (115.1±41.6 mg/dL).
Conclusion: Our study supports the hypothesis that overweight is a risk factor not only for younger and middle-aged, but also for active elderly patients. Fasting glucose levels revealed a continuous increase up to the oldest age groups. Preventive strategies for type 2 diabetes should thus be offered for all age groups.

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Comparison of visceral adiposity and liver fat in 4277 individuals from an international cohort of patients classified according to their glucose tolerance status: the INSPIRE ME IAA Study

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Background and aims: Excess visceral adiposity and liver fat are well known correlates of metabolic abnormalities increasing the risk of type 2 diabetes (T2D) and cardiovascular disease. The first aim of the INSPIRE ME IAA study was to determine the relationships between visceral adiposity and liver fat measured by computed tomography (CT), related cardiometabolic risk markers and history of ischemic cardiovascular events and T2D.
Materials and methods: The INSPIRE ME IAA study (INternational Study of Prediction of Intra-abdominal adiposity and its RElationships with cardioMEtabolic risk/Intra-Abdominal Adiposity) is an international observational prospective study with a planned 3-yr follow up conducted in 29 countries and involving 297 physicians. Physicians were either 1- hospital based primary care physicians and internists, 2- cardiologists or 3- endocrinologists/diabetologists with approximately 1/3 of each clinical practice group represented. Of the 4505 patients included in the study, 4277 had data available for the present analyses. Male outpatients were aged 40-70 years whereas female outpatients were aged 45-70 years. At baseline, demographic and clinical data were obtained as well as a cardiometabolic profile which included a 75 g oral glucose tolerance test. Visceral adiposity and liver fat were measured by CT and all images were read centrally by a core imaging laboratory. The present analyses focus on the comparison of visceral adiposity/liver fat across subgroups of patients defined according to their glucose tolerance status: 1- normal (NGT), 2- impaired fasting glucose (IFG) or impaired glucose tolerance (IGT), 3- controlled T2D (HbA1c<7%), 4- poorly controlled T2D (HbA1c≥7%). All comparisons across the 4 groups were adjusted for age, region and clinical practice group.
Results: Across the 4 groups with differing glucose control, there was a clear gradient for waist circumference with the lowest values found in NGT subjects and the highest in poorly controlled T2D patients (p<0.001). Accordingly, there was also a positive gradient for fasting triglyceride levels and a negative gradient for HDL-cholesterol, the lowest values being found in patients with poorly controlled T2D (p<0.005). There was also a progressive increase in visceral adiposity measured by CT from the NGT subjects to the poorly controlled patients with T2D (p<0.001). Mean attenuation value of the liver (used as an index of liver fat) also showed significant group differences (p<0.001). In men and women, significant correlations were observed between visceral adiposity and the CT-derived index of liver fat (r=-0.39 in men and r=-0.47 women, p<0.001).
Conclusion: INSPIRE ME IAA is the largest international study on visceral adiposity/liver fat and cardiometabolic risk profile. Analyses of the baseline data revealed marked differences in visceral adiposity/liver fat associated with glucose tolerance status. In both men and women, T2D diabetes is characterized by high levels of visceral adipose tissue/liver fat. A strong association between visceral adipose tissue and liver fat is found in both men and women. Excess visceral adiposity/liver fat is associated with a deteriorated cardiometabolic risk profile in patients with T2D.
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Variants in vitamin D genes are associated with liver density and non-alcoholic fatty liver disease (NAFLD) in Hispanics and African Americans: the IRAS Family Study

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Background and aims: NAFLD is a condition that may be involved in the pathogenesis of type 2 diabetes, obesity, and the metabolic syndrome. Given the association between these factors and vitamin D status, we examined whether vitamin D pathway genes are associated with NAFLD in Hispanic- (HA) and African-Americans (AA), two ethnicities at increased risk for obesity, type 2 diabetes, and vitamin D deficiency.
Materials and methods: After eliminating 120 subjects with excessive alcohol intake, the IRAS Family Study examined 830 HA and 350 AA individuals from families in San Antonio, San Luis Valley and Los Angeles who had a computed tomography (CT) measure of the liver and visceral fat (VAT), and single-nucleotide polymorphism (SNP) data on *VDR* (15 SNPs), *DBP* (11 SNPs), *CYP27B1* (5 SNPs), *CYP24A1* (22 SNPs), and *CYP2R1* (3 SNPs) genes. The continuous outcome liver density was examined using a variance components approach, while the dichotomous outcome of NAFLD (liver:spleen density ratio < 1) was examined using generalized estimating equations (GEE); both analytic approaches account for the relatedness of the subjects. Additive models were

assumed for the SNPs. Models were adjusted for age, gender, admixture, and VAT. HA analyses were also adjusted for clinic site.
Results: In HA, mean age was 48.2 years, 63.4% were female, and mean VAT was 116.9 cm^2. In AA, mean age was 49.8 years, 60.2% were female, and mean VAT was 103.3 cm^2. 236 subjects were classified as having NAFLD (203 HA and 33 AA). *CYP27B1* and *CYP2R1* were not associated with liver density nor NAFLD in HA and AA. In HA, *DBP* and *VDR* were not associated with NAFLD, however, rs4334089 in *VDR* in was associated with liver density (p=0.027). In AA, while *DBP* and *VDR* were not associated with liver density, 3 SNPs in *DBP* and 1 SNP in *VDR* were associated with NAFLD. In AA, each copy of the A allele at rs10783219 in *VDR* resulted in 3.70 greater odds of having NAFLD (95% CI: 1.03-14.29); and for *DBP*, each copy of the T allele at rs4752 resulted in 3.09 greater odds of NAFLD (95% CI:1.51-6.34); each copy of the C allele at rs222020 resulted in 1.89 greater odds of NAFLD (95%CI: 1.08-3.33); and each copy of the G allele at rs10011000 resulted in1.96 greater odds of NAFLD (95% CI: 1.13-3.40). Several SNPs in *CYP24A1*were associated with liver density in AA [rs2248359 (p=0.036), rs3787554 (p=0.023), rs4809960 (p=0.044), rs6022999 (p=0.016)] and in HA [rs3787555 (p=0.013), rs6068816 (p=0.002), rs6097809 (p=0.029), rs6127119 (p=0.039)]. In addition, SNPs rs3787557 in AA and rs6068816 in HA were significantly associated with NAFLD. In AA individuals, each copy of the C allele at rs3787557 resulted in 2.60 greater odds of having NAFLD (95% CI: 1.33-5.07). In HA individuals, each copy of the C allele at rs6068816 resulted in 1.59 greater odds of having NAFLD (95% CI: 1.05-2.38).
Conclusion: In a two minority populations at increased risk for obesity and type 2 diabetes, variants in vitamin D pathway genes, particularly *CYP24A1*, are associated with liver density and NAFLD.
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Using imputation to investigate association of low-frequency variants with adiposity in the 1966 Northern Finnish birth cohort
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Background and aims: Adiposity measures are known to be substantially heritable. While genome-wide association studies (GWAS) have identified over 20 common variants associatied with adiposity measures, less than 3% of the total heritability of adipose traits has been explained. One possible source of this missing heritablity is in low-frequency causal variants, say with minor allele frequency (MAF) <5%. Such variants pose several analytical challenges, due to a lack of statistical power for detecting association and their sparse availibility on current GWAS genotyping chips (by design). Here, we utilise genotype imputation and a novel statistical test of association to try and overcome these challenges.
Materials and methods: Given a set of SNPs for some individuals, imputation allows us to estimate genotypes at untyped variants in each individual by utilising a combination of population reference panels and a fine-scale recombination map. The recent release of 1000 Genomes pilot data (and already available HapMap3 data) allows us to impute the majority of low-frequency variants with MAF as low as 1%, as well as many much rarer variants. Using this newly available data, we test for association of gene regions with adiposity measures by considering the proportion of low-frequency and rare variants per genomic region at which individuals carry minor alleles in a linear regression framework. This method offers improved power over the traditional tests of association typically applied to common variants. Using this framework, four different adiposity phenotypes were investigated in the 1966 Northern Finnish Birth Cohort (genotyped using the Illumina HumanCNV-370DUO chip). We examined variants with MAF of <5% for association with BMI, hip circumference (HC), waist circumference (WC) and waist to hip ratio (WHR). Additional covariates were included in the model to adjust for population structure as well as sex. Models were also fitted separately for each sex.
Results: Imputation allowed us to investigate 89 514 and 1 121 653 variants with MAF <1% and <5% respectively, as opposed to the original GWAS data which contained 14 119 and 28 470 variants. The strongest signal of association over all genes and phenotypes was with WHR adjusted for BMI in males on a region of chromosome 7 ($p=2.1x10^{-8}$, allelic effect β=-0.23 [-0.31, -0.15]). In addition to this, a total of nine genes demonstrated strong evidence of association ($p<10^{-5}$) with the adiposity measures investigated. A further ten genes with nominal evidence of association ($p<10^{-4}$), but with plausible biological roles in adiposity have been highlighted for further investigation. For example, three genes on chromosome 11; PNPLA2, RPLP2 and TSPN4, had $p<10^{-4}$ and are all reported to be preferentially expressed in adipose tissue.
Conclusion: A number of novel possible adiposity associated genomic regions have been identified for follow up. Many of the identified regions have previously been identified as having plausible biological roles in adiposity and these regions are currently being sequenced in individuals at the extremes of the phenotypes.
Supported by: WT

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***MC4R* gene variant rs17782313 (C/T) is associated with increased muscle mass in lean women**
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Background and aims: Melanocortin-4 receptor (*MC4R*) plays a critical role in regulating food intake and energy balance. MC4R activation in brain reduces food intake and increases energy expenditure via leptin signaling. MC4R deficiency is the most common form of monogenic obesity. Recently, the meta-analysis of 15 genome-wide association studies strongly confirmed the association of the common polymorphism rs17782313 lying 188 kb downstream of the *MC4R* gene with body mass index (BMI) and obesity risk in adults and children. We examined the impact of the rs17782313 (C/T) on obesity in cohort of Czech women and studied its possible metabolic effects.
Materials and methods: Polymorphism was assesed by ABI TaqMan SNP Genotyping Assay in 951 non-diabetic normoglycaemic women: 128 lean (age 26.4±5.86 years; BMI 18.9±0.92 kg/m^2), 409 normal weight (age 30.4±9.84 years; BMI 22.3±1.38 kg/m^2), 208 overweight (age 37.8±13.65 years; BMI 27.3±1.39 kg/m^2) and 206 obese women (age 38.6±13.86 years; BMI 34.9±4.11 kg/m^2). All women were detailed anthropometrically and biochemically characterized including oGTT and ITT tests. For statistical analyses the Mann-Whitney test and Chi-square test were used (NCSS 2004).
Results: Genotypes were in Hardy-Weinberg equilibrium. The allelic frequencies did not differ among groups (risk minor C allele: lean 33.2%; normal 27.3%; overweight 26.9%; obese 29.1%). The frequency of the minor allele corresponds to the other European populations. In the whole group of pooled women (age 30.5±9.28 years; BMI 24.9±5.54 kg/m^2), the carriers of the minor C allele (CC+CT) did not differ in BMI in comparison with non-carriers (TT) and surprisingly, they had significantly lower WHR (0.82 vs. 0.84, p=0.046) and waist circumference (73.8 vs. 75.9 cm, p=0.035) and higher serum creatinine level (69.9 vs. 66.9 umol/L, p=0.005). These effects were most apparent in the subgroup of lean women with BMI<20 kg/m^2 where the C allele carriers had significantly lower WHR (0.78 vs 0.82, p=0,001), waist (65.6 vs. 66.9 cm, p=0.02) and abdominal circumference (69.5 vs. 74.9 cm, p=0.008), higher % of muscle mass (41.3 vs. 39.1, p=0.0001) and higher creatinine levels (73.4 vs. 64.2 umol/L, p=0.02). In the sugroup of obese women with BMI > 30 kg/m^2, the association of the C allele with anthropometric parameters was not found, but the C allele carriers tend to be more obese and had also increased creatinine levels compared to non-carriers (69.8 vs. 61.9 umol/L, p=0.01).
Conclusion: We did not confirm the association of rs17782313 with obesity in our cohort of women. However, the C allele carriership was associated with increased creatinine levels and increased % of muscle mass, especially in lean women.
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The effect of birth weight on obesity is not modified by *FTO* rs9939609
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Background and aims: Increased birth weight and the *A* allele of *FTO* rs9939609 are both associated with adult obesity. However, it is unknown

whether the effects of birth weight and *FTO* variation are additive or whether the *FTO* risk variant modifies the effect of birth weight on adult obesity. Thus, the aim of this study was to examine whether there is an interaction between birth weight and the *FTO* rs9939609 on the development of adult obesity.
Materials and methods: Baseline data from the Danish population-based Inter99 study were used. Birth weight data on 4,584 participants (all singletons) were collected through midwife journals from the Danish State Archives. The *FTO* rs9939609 was genotyped using KASPar® technology (n=4,371). Obesity was assessed by body mass index (BMI). Age- and sex-adjusted linear regression analyses with BMI as outcome were performed. Model 1 allowed for interaction between birth weight and *FTO* rs9939609, whereas model 2 only included main effects of birth weight and *FTO* rs9939609.
Results: Mean BMI in the population was 26.11 kg/m^2 (SD: 4.5), mean age was 46.3 years (SD: 7.9), and 46.5% were men. There was no interaction between birth weight and the FTO rs9939609 variant on adult BMI (P=0.23, model 1), but both birth weight and FTO rs9939609 were independently associated with adult BMI in model 2. One kg increase in birth weight was associated with an increase in adult BMI of 0.46 kg/m^2 (95% CI: 0.20-0.71, P<0.001). Likewise, the *A* allele of FTO rs9939609 was associated with an increase in BMI of 0.49 kg/m^2 (95% CI: 0.30-0.68) per risk allele (P<0.001) assuming an additive genetic model.
Conclusion: The effect of birth weight on obesity in adult life is not modified by the FTO rs9939609 variant in the Danish population. Thus, *FTO* variation and birth weight contribute independently to adult obesity.
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GIP receptor polymorphism rs10423928 affects body mass index and insulin and glucagon response after ingestion of glucose or mixed meals in Japanese

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Background and aims: GIP enhances insulin and glucagon secretion, and regulates fat deposition and bone formation through binding to GIP receptor (GIPR). Recent study showed an association of a single nucleotide polymorphism (SNP) in the human GIPR gene, rs10423928 with elevated post-challenge plasma glucose levels. To evaluate effects of this SNP, as well as SNPs in KCNQ1 and TCF7L2, on secretion of insulin and glucagon, we measured levels of insulin, glucagon and glucose in response to ingestion of glucose or mixed meal in Japanese untreated type 2 diabetes (T2DM), impaired glucose tolerance (IGT) and healthy controls.
Materials and methods: Japanese healthy controls (n=33, age 46±;2, HbA1c 5.2 ±;0.0%, BMI 21.6±;0.5), IGT (n=24, age 56±;2, HbA1c 5.9±;0.3%, BMI 23.7±;0.5) and untreated T2DM (n=22, age 58±;2, HbA1c 6.3±;0.1%, BMI 23.3±;0.4; duration 1.9±;0.4 years) were subjected to 75-g oral glucose and 480-kcal meal tolerance tests (OGTT and MTT, respectively) and their glucose, insulin and glucagon levels were measured. SNPs were genotyped by an allele-specific primer PCR method using peripheral leukocyte DNA of each subject.
Results: Allele frequencies of SNPs in each group were as follows: GIPR rs10423928 Controls, AA 0.2/AT0.3/TT0.5; IGT, AA0/AT0.3/TT0.7; T2DM, AA0/AT0.4/TT0.6; KCNQ1 rs2237892 Controls, CC0.4/CT0.4/TT0.1; IGT, CC0.4/CT0.4/TT0.2; T2DM, CC0.4/CT0.4/TT0.2; TCF7L2 rs7903146 Controls, CC0.9/CT0.1/TT0; IGT, CC0.9/CT0.1/TT0; T2DM, CC0.9/CT0.1/TT0. Parameters that are significantly different (unpaired t-test, p<0.05) between GIPR rs10423928 AA- and TT-carriers are as follows (Figure 1): BMI, glucose-AUC(0-120) in OGTT and MTT, and glucagon-AUC(0-120)in OGTT and MTT. Parameters related to insulin secretion (i.e. insulinogenic index, insulin-AUC(0-120), HOMA-beta) and insulin resistance (i.e. HOMA-IR) show no significant difference between AA- and TT-carriers, although insulin-AUC(0-120) in OGTT and MTT showed significance difference between AT- and TT-carriers. No parameter related to insulin and glucagon secretion shows significant difference among KCNQ1 rs2237892 CC-, CT- and TT-carriers. No parameter shows significant difference between TCF7L2 rs7903146 CC- and CT-carriers.
Conclusion: GIPR rs10423928 AA-carriers have significantly lower BMI, reduced glucagon secretion, and lower plasma glucose levels after ingestion of glucose or meal. In addition, GIPR rs10423928 also affects insulin secretion after ingestion of glucose or meal. Notably, no GIPR rs10423928 AA-carrier was found in IGT and T2DM of the current study. Although the subject number in the current study is limited, our results are consistent with roles of GIP in secretion of glucagon and insulin and fat accumulation, and strongly suggests that GIP dysfunction could play a role in pathogenesis of T2DM.

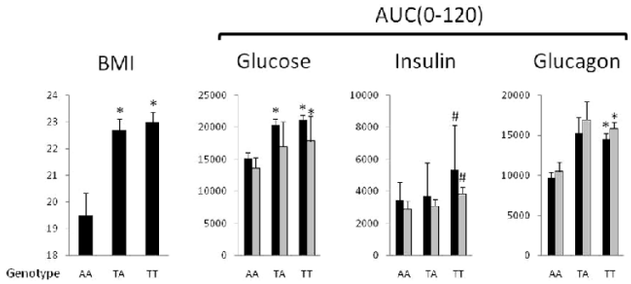

Figure 1. Effects of GIPR SNP on BMI and Levels of Glucose, Insulin and Glucagon. Mean±SEM. OGTT, black bars and MTT, gray bars.
* and #, Significant difference (unpaired t-test , p<0.05) versus AA and TA, respectively.

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Replication initiator 1 gene (*Repin1*) is involved in the pathophysiology of human obesity

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Background and aims: The replication initiator 1 gene (*Repin1*) maps within a quantitative trait locus for obesity and is related to dyslipidemia in subcongenic rat strains. Here, we investigated the role of *Repin1* in the pathophysiology of human obesity.
Materials and methods: *Repin1* mRNA expression was measured in intraabdominal visceral (Vis) and abdominal subcutaneous (Sc) adipose tissue in 196 individuals with a wide range of metabolic phenotypes using RT-PCR (TaqMan, Applied Biosystems, Inc.). The *Repin1* was sequenced (exons, exon-intron boundaries, 5´and 3´ UTRs) in DNA samples from 48 non-related Caucasian subjects to identify genetic variants. 18 variants were identified, including a 12 bp deletion in exon four resulting in a final protein missing four amino acids (rs3832490; P356_A359del). The deletion and nine single nucleotide polymorphisms (SNPs) including six HapMap (www.hapmap.org) tagging SNPs representing their linkage disequilibrium groups were genotyped for subsequent association studies in two independent cohorts with detailed metabolic testing: German Caucasians from Leipzig (N=2194; mean age 56±15 years) and a self-contained population of Sorbs from Germany (N=1046; 48±16 years), totalling 3240 subjects. TaqMan assays were used for SNP genotyping and restriction fragment length polymorphism (RFLP) technique for the deletion.
Results: We found significant correlations between *Repin1* mRNA expression in human Vis and Sc adipose tissue and total body fat mass as well as adipocyte size, suggesting *Repin1* as novel candidate gene for human obesity and related traits. In a case control study including 1018 subjects with type 2 diabetes (T2D) vs. 616 subjects with normal glucose tolerance (NGT), three SNPs (rs3735170, rs10278590, rs1051760) were significantly associated with T2D (P<0.05 after adjusting for age, sex and BMI) in the Leipzig cohort. In subjects with NGT, rs4725336 was significantly associated with cholesterol, rs9640161 with HbA1c, and rs3832490 (P356_A359del) with % body fat and 2 hr glucose (adjusted P<0.05). In the self-contained population of the Sorbs, rs4725336 showed association with obesity in a case control study including 397 obese (BMI>30 kg/m^2) vs. 234 lean (BMI<25 kg/m^2) subjects (adjusted P<0.05). Consistent with results from the Leipzig cohort, rs3832490 (P356_A359del) was moderately associated with % body fat in Sorbian subjects with NGT (N=835) and rs6971465 correlated with cholesterol and LDL-cholesterol (adjusted P<0.05).
Conclusion: Correlation of mRNA expression in adipose tissue with obesity as well as the association of *Repin1* genetic variants with T2D, obesity and relevant metabolic traits suggest a potential role of *Repin1* in the pathophysiology of human obesity.

PS 9 Epidemiology of type 1 diabetes mellitus: incidence and mortality

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Estimate of incidence and prevalence of type 1 diabetes using electronic drug prescription archives

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Background and aims: A very wide range of childhood diabetes incidence rates within Europe has been shown, but no recent data are available in Italy. Type 1 diabetes features are that is usually diagnosed in children and young adults, and causes dependence on insulin treatment for life. On the other hand, diabetes is the only indication for insulin therapy. Aim of this study was to estimate incidence and prevalence of type 1 diabetes among people 0-15 years old in the Lazio Region in the period 2005-2008, and to describe the insulin prescription pattern, using a record linkage between drug prescription and National Health Service (NHS) enrollee electronic archives.
Materials and methods: The Italian NHS provides antidiabetic drugs free of charge to all citizens. Data on outpatient antidiabetic drug use were obtained from the Regional drug prescription monitoring database for the period 2005-2008. Data on characteristics of population exposed to the study drugs were derived from Lazio (about 6 millions inhabitants) database of NHS enrollees, which contains demographic data of residents. The two databases can be linked by a unique individual code allowing to trace back an hystorical patient drug profile. Patients were considered as "prevalent" cases if they received insulin (ATC A10A - insulins and analogs) prescriptions during the study period. "Incident" case was defined as a patient who received the first insulin prescription in the period 2006-2008, without any antidiabetic prescription in the previous 12 months. The date of the first insulin prescription was used as date of diagnosis.
Results: An annual mean of 692 type 1 diabetes cases were identified in the four-year study period. The prevalence of diagnosed type 1 diabetes increased from 81 (CI 95% 74-87) per 100,000 inhabitants in 2005 to 91 (CI 95% 84-97) in 2008 with no evidence of a difference between boys and girls. The prevalence rates increase with age, in 2008 they were 20 per 100,000 (CI 95% 11-28) for children aged 1-3 years, and 173 (CI 95% 156-191) for 12-15 years. A total of 469 incident cases were identified from 2006 through 2008. The cumulative incidence varied from 21 (CI 95% 17-24) per 100,000 in 2006 to 17 (CI 95% 14-19) in 2008. At the first prescription, the majority of children received rapid-acting human insulin and analogs. Overall, with respect to 2005 there was a shifting of prescriptions to long-acting insulin.
Conclusion: The estimated incidence is quite higher from that previously reported for Lazio: 8.1 per 100,000 in the period 1993-94. Our study shows a limited increase in the prevalence rates of diagnosed type 1 diabetes among children 0-15 years from 2005 through 2008. However, the study period was too short to investigate a time trend in incidence and prevalence. Completeness of ascertainment is being evaluated through a validation procedure with the data of one of the most important diabetic centre of the Region. The use of routinely collected data to estimate incidence and prevalence of type 1 diabetes, and to identify cohorts of patients, may represent an alternative to other costly and time consuming methods.

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Increasing incidence of childhood onset type 1 diabetes in Norway

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Background and aims: There is a worldwide increase of type 1 diabetes (T1DM). In 1989, the Norwegian population-based childhood diabetes register was initiated including all newly diagnosed children aged 0-14 years with diabetes. All 26 paediatric departments in Norway reports new cases of childhood diabetes to The Norwegian Childhood Diabetes Register based on informed consent from the child and/or parents. Since 2004 the Prescription Database (NorPD) at the National Institute of Public Health has registered all drug prescription in Norway, including insulin. Data published by the authors previously showed a clear increasing trend during the period 1973 - 2003 with IR moving from 19.1/10^5 in 1973 to 28.9/10^5 in 2001-2003. The aim of the study was to determine the incidence of T1DM in children 0-14 years in Norway during 2005-2008, and to calculate the ascertainment in the nationwide Norwegian Childhood Diabetes Register during the same period.
Materials and methods: During the study period 2005-2008,1232 new cases of childhood onset diabetes were registered by The Norwegian Childhood Diabetes Register. Of these, 1144 were classified as T1DM and were below 15 years at onset. Information on individual insulin prescriptions was obtained from The Prescription Database, and the first prescription of insulin 2004-2008 was registered and assumed to be at a date close to onset of T1DM. Consequently, "new cases" in the NorPD could only be defined for the years 2005-2008. The assumption of source independence and equal probability of capture of each case by these two sources is verifiable. This is the first time these two registries are linked with the purpose to give information about incidence of childhood T1DM and completeness of The Norwegian Childhood Diabetes Register.
Results: In the period 2005-2008, the uncorrected incidence rate of T1DM 0-14 years was 32.4 /10^5/year for both sexes, for boys 34.1*10^5 and for girls 30.9*10^5, which indicates an steadily increasing trend compared to previously published incidence rates. The Prescription Database contained data on first time insulin prescriptions in 115 subjects, not reported to the diabetes register and the completeness is calculated to 92 % for the whole study period which is appropriate according to the criteria for entering national data into the EURODIAB study.
Conclusion: The incidence of type 1 diabetes has further increased in Norway as in many other European countries. The incidence has been studied nationwide since 1989 and the completeness of registration of new cases can be documented to be 90-95% % in all periods with obligatory signed informed consent forms from all patients.

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The influence of birth cohort on the incidence of childhood type 1 diabetes in Wales

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Background: It is suggested that type 1 diabetes (T1DM) occurs when genetically susceptible individuals experience a viral infection in the period preceding diagnosis: a period effect. There is also evidence that perinatal factors influence the incidence of T1DM in childhood. Systematic change in these would produce a birth cohort effect meaning an effect from being born at a particular time or having shared experience with those born at the same time.
Aims: To examine the influence of period and birth cohort on the incidence of T1DM in children aged <15y in Wales from 1995-2008.
Materials and methods: Information on 2046 cases diagnosed with T1DM from 1995-2008 was obtained prospectively from all paediatric units in Wales. Ascertainment was >95% in each calendar year to 2006.
Results: The overall increase in incidence (poisson regression modelling) assuming continuous linear growth was 1.9% per annum (residual deviance 0.84, $p<0.001$) but best fit was obtained with a model showing no further increase in incidence rate since 2000 (residual deviance <0.7, $p<0.001$) with no further increase in cases before age 5. The current annual incidence rate is 28 cases per 100,000 children. Modelling the influence of birth cohort suggests an effect of approx 1.5 on incidence rate when birth cohorts since 1985 are compared with 1980-84 and that this effect has not increased since 1985. Adding period effect produces a less good model (residual deviance 1.27, $p<0.001$) but indicates that an increasing period effect is counterbalanced by a reducing birth cohort effect.
Conclusion: The incidence rate of T1DM has not increased in Wales since 2000. Birth cohort effects have more influence than period effects and the absence of any continuing increase in birth cohort effect in Wales is a major influence on the incidence rate. Perinatal factors therefore require further investigation. Differences in the relative contribution of birth cohort and period effects on incidence compared to other nations may explain the current plateau in incidence in Wales.

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Incidence of childhood and youth type 1 diabetes in La Palma Island 1993-2009

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Background and aims: The incidence of type 1 diabetes shows wide geographical variability and heterogeneity. The aim of this study was to determine the incidence of type 1 diabetes in children and young people younger than 30 yr in La Palma Island (the most northwest of Canary Islands, Spain: 730 Km^2, 85000 habitants and subtropical climate).
Materials and methods: All subjects with type 1 diabetes (according WHO and/or ADA criteria) diagnosed between January 1993 and December 2009 (prospectively 1995-2009) were included. The population at risk (0-29 yr) fluctuated between 36419 habitants (15711 habitants younger than 15 yr) -1991 General Census- and 29620 (11899 habitants younger than 15 yr) -2001 General Census-. All the reported cases were on insulin treatment. All subjects were living in La Palma Island at least six months before diagnosis of type 1 diabetes. Using the capture-recapture method (primary source was hospital records, while secondary sources were membership files of La Palma Diabetic Association and Primary Care Physicians), the ascertainment was 100 %. The incidence rates were expressed as number of cases per 10^5 habitants per year. The 95% Confidence Intervals were estimated assuming the Poisson distribution of the cases. The age adjustment for the rates was done using the direct method with a World and European Standard Population.
Results: 113 subjects younger than 30 yr had presented type 1 diabetes at the last 17 yr (64 boys, 49 girls; medium age: 13±7.6 yr). The annual incidence fluctuates between 5.8 and $33.7/10^5$, being the average annual incidence $20.8/10^5$ (95% CI: 18.6-23; $34/10^5$ in the 0-14 yr group, CI 95%: 31.2-36.8; $11.6/10^5$ in the 15-29 yr group, CI 95%: 10-13.2) without sex differences ($21.4/10^5$ per yr in boys versus $17.3/10^5$ per yr in girls). The incidence was higher in the 5-9 yr age-group ($39.6/10^5$ per yr), followed by the 10-14 yr age-group ($38.4/10^5$ per yr), the 0-4 yr age-group ($21.9/10^5$ per yr), the 25-29 yr age-group ($13/10^5$ per yr), the 15-19 yr age-group ($12.8/10^5$ per yr) and the 20-24 yr age-group ($9/10^5$ per yr). The age-adjusted incidence to World Standard Population was $23.1/10^5$ per yr (95% CI: 20.8-25.4; $32.4/10^5$ in the 0-14 yr group, $11.6/10^5$ in the 15-29 yr group]. The age-adjusted incidence to European Standard Population was $22.4/10^5$ per yr (95% CI: 20.2-24.6; $32.8/10^5$.in the 0-14 yr group, $11.6/10^5$ in the 15-25 yr group).
Conclusion: The incidence of type 1 diabetes in La Palma Island is the highest reported up to date in a Spanish community, and is close to the highest of the world. It is inconsistent with the hypothesis of a north-south gradient in diabetes risk. The knowledge of the incidence rates in La Palma Island can contribute to study the role that genetics and environmental factors may play in these differences.

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Prospective nation wide registration of type 1 diabetes incidence in 0-34 years old during 25 years - a shift to younger age at diagnosis

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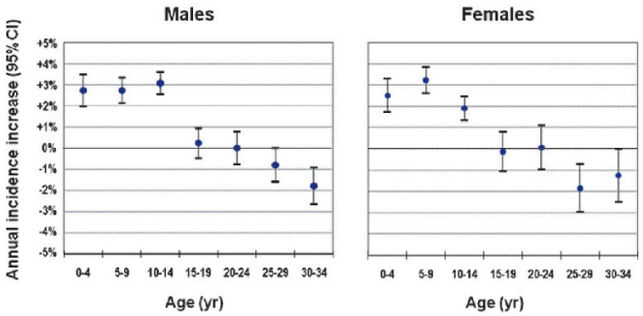
Background and aims: To clarify if the increase in childhood type1 diabetes is mirrored by a decrease in older age groups on a birth cohort basis and to test the hypothesis that autoimmune diabetes shifted to younger age at onset. To test if cohort effects dominated over calendar period effects.
Materials and methods: The data base included 20 249 individuals with diabetes onset 1983 to 2007 combining data from two prospective research registers, the Swedish Childhood Diabetes Register including cases 0-14.9 years at onset and the Diabetes in Sweden Study including cases 15-34.9 years at onset. Incidence rates over time were analyzed using Poisson regression models.
Results: The overall yearly incidence rose to a peak of 42.3 per 100000 in males10-14 years and of 37.1 in females 5-9 years age group and decreased thereafter with increasing age. There was a significant increase by calendar year in both sexes in the three under 15 years' age groups but decreases in the older age groups with significant decreases in the two age groups over 25.A cohort effect dominated over the time period effect.
Conclusion: Twenty-five years of prospective nationwide incidence registration demonstrates a clear shift to younger age at onset rather than a uniform increase in incidence rate across all age-groups. Exposures that affect young children more than older children and adults and accelerates disease onset may be responsible.



Supported by: Swedish Research Council

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Ethnic differences in incidence rates of childhood type 1 diabetes in Yorkshire 1978-2007

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Aims: To examine incidence rates and trends of childhood Type 1 diabetes in Yorkshire from 1978-2007.
Materials and methods: Data from the population-based Yorkshire Register of Diabetes in Children and Young People was used to analyse the incidence of Type 1 diabetes in children aged <15 years diagnosed in the former Yorkshire Regional Health Authority. Incidence rates (per 100,000 per year) were estimated using mid-year population estimates stratified by sex, age and ethnicity: south Asian (Indian, Pakistani, Bangladeshi) or non-south Asian (all other ethnicities). Ethnicity was assigned using two name recognition programs (Nam Pehchan and SANGRA) and a local expert. Age-sex standardised rates were calculated between 1978-2007 and by ethnic-group between 1990-2007. Poisson regression was used to assess incidence trends and estimate predicted rates up to 2020. Goodness-of-fit, AIC and likelihood-ratio tests were used to assess model fit.
Results: 3911 children were diagnosed in Yorkshire between 1978-2007. Overall incidence was 18.1 (17.6-18.7) and lowest among 0-4 year-olds compared to 5-9 and 10-14 year-olds: 11.7 (95% CI 10.9-12.5), 18.6 (17.6-19.6) and 23.7 (22.6-24.8) respectively. Incidence increased significantly over time with an average annual percentage change (AAPC) of 2.9% (2.5-3.2). The AAPC differed slightly between age-groups - 0-4: 2.6% (1.7-3.4), 5-9: 3.2% (2.6-3.8) and 10-14 years: 2.8% (2.2-3.3). The inclusion of an age-sex interaction term provided evidence for differences in trends between sexes depending on age, with females having higher incidence and AAPC than males for those aged 5-9. Incidence for non-south Asians (21.6 (20.7-22.4)) was significantly higher than that of south Asians (14.7 (12.4-17.1)) over the entire study period. A significant increasing trend in incidence was observed for non-south Asians of 3.4% (2.7-4.2) compared to a non-significant trend seen in south Asians (1.5% (-1.5, 4.5)). Overall forecasted incidence for 2020 is 38.8.
Conclusion: Type 1 diabetes incidence rates have continually risen over the last three decades for non-south Asians of all ages but not for south Asians, whose families come from a low incidence area. This is contrary to findings in the Bradford area of Yorkshire between 1978-1998. Overall incidence increased most quickly in the 5-9 age-group. Incidence doubled from 12.5 to 25.6 between 1978-2007. If current trends continue, rates will rise by 52% to 38.8 between 2007-2020.
Supported by: NHS Diabetes, HCQIP

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Long-term mortality and causes of death among patients with type 1 diabetes in Japan

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Aims: To investigate long-term prognosis including causes of death among patients with type 1 diabetes in Japan.
Methods: A total of 1,387 patients (556 males and 831 females) were registered from two nationwide type 1 diabetes surveys in Japan. They were diagnosed as type 1 diabetes at less than 18 years of age between 1965 and 1979. All patients were tracked for survival status until January 1, 2005, with this status determined based on the questionnaires sent to their attending physicians or the residents' records. Causes of death were identified through questionnaires or death certificates. Their survival status as of January 1, 2005 was expressed in terms of standardized mortality ratio (SMR) and crude mortality rate (CMR). Mortality was compared between the male and female patients by using the Cox proportional-hazards model. The causes of death for deceased cases were divided into 9 groups (1. diabetic renal disease; 2. acute diabetic complications; 3. accident/suicide; 4. cardiovascular disease; 5. infections; 6. malignant neoplasms; 7. other non-diabetic causes; 8. other diabetic causes; 9. unknown) and were also compared by duration of diabetes. Statistical analyses were performed by using SAS 9.1.
Results: The mean age at diagnosis was 8.8 ± 4.1 (SD) years, with a duration of diabetes of 27.9 ± 6.4 years. One thousand one hundred and three patients were confirmed as alive as of January 1, 2005, and 223 deaths (16.1%) were observed (confirmation rate: 95.6%). The SMR was 10.6 (males, 9.6; females 14.3), and the CMR was 658/100,000 person-years (males, 778; females, 579). The male patients were shown to be at 1.37-fold higher mortality risk compared to the female patients (95% CI, 1.02-1.85). The causes of death identified included diabetic renal disease (51 patients; 22.9%), cardiovascular disease (40; 17.9%), acute diabetic complications (38; 17.0%), infections (34; 15.3%), accidents and suicides (21; 9.4%), unknown cause (18; 8.1%), other non-diabetic causes (13; 5.8%), other diabetic causes (6; 2.7%), and malignant neoplasms (2; 0.9%). Leading causes of death included acute diabetic complications among those with less than 10 years' duration of disease, diabetic renal disease among those with 10 to 20 years' duration, infections among those with 20 to 30 years' duration, and cardiovascular disease among those with 30 to 40 years' duration. Thus, the longer the duration of disease, the less the mortality from acute diabetic complications, and the greater the mortality from cardiovascular disease. The time from the initiation of dialysis to death was shown to be 5.5 ± 4.8 years in 89 patients who were confirmed to have been dead after initiation of dialysis.
Conclusion: The mortality risk of patients diagnosed as type 1 diabetes between 1965 and 1979 in Japan was shown to be 10.6-fold higher than that of the general population. The males were found to be at 1.37-fold higher mortality risk than the females. Diabetic renal disease, cardiovascular disease and acute diabetic complications were found to be the leading causes of death. However, as the duration of disease became longer, acute diabetic complications contributed less and cardiovascular disease contributed more to mortality.
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PS 10 Environmental factors and type 1 diabetes mellitus

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First trimester cytokine levels in mothers to children diagnosed with islet autoimmunity or type 1 diabetes before eight years of age

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Background and aims: Gestational infections and/or stress have with conflicting results been associated with an increased type 1 diabetes risk in offspring. Using multi-array analysis of cytokines, we tested whether Th1/Th2 cytokines during the first trimester were different between mothers who gave birth to children who developed islet autoimmunity at 1-8 years of age and matched control mothers.
Materials and methods: First trimester serum samples were analyzed for IFNγ, IL-10, IL-12, IL-13, IL-1β, IL-2, IL-4, IL-5, IL-8 and TNFα using the Meso-Scale Multi-Array system (MesoScale, Gaithersburg, Maryland). We compared 53 non-diabetic mothers who gave birth to a child who developed at least two islet autoantibodies against either GAD65, IA-2 or insulin with increasing levels at the second, third, fourth or fifth year of follow-up (a total of 40 children developed type 1 diabetes before 8 years of age) with 106 non-diabetic control mothers which were matched by age, HLA genotype as well as first trimester sampling date. The mothers included in this study are participating in the Skåne Diabetes Prediction Study (DiPiS). In 2000-2004, DiPiS recorded 48 058 live births with the aim to determine etiology indicators of type 1 diabetes. Blood samples (both mothers serum and cord blood) were obtained at the time of delivery in 75% of the 48 058 recorded live births, and first trimester samples (10-16 gestational weeks) were available from the Southern Sweden Microbiological biobank for the non-diabetic mothers included in this part of the study.
Results: The median of IFNγ (p=0.02) and IL-1β (p=0.04) levels were significantly higher in the index mothers compared to the matched controls. The mean length of gestation in the index mothers was 275 days compared to 280 days in control mothers (p=0.04). The shortened gestational length was not related to the IFNγ or IL-1β levels. However, the gestational length in index but not control mothers was significantly correlated to IL-10 (p=0.03), IL-12 (p=0.01), IL-13 (p=0.04), IL-2 (p=0.04) and IL-5 (p=0.008).
Conclusion: This study revealed that 1) index mothers had elevated Th1 mediated cytokines (IFNγ and IL-1β) during the first trimester; 2) gestational length was significantly shortened in the index mothers; and that 3) several Th2 mediated cytokines were inversely related to the gestational length in the index but not in the control mothers, in first trimester samples. We therefore conclude that an increase in Th1 cytokine levels during the first trimester may signify gestational infection or stress. Furthermore, we conclude that Th2 cytokines may affect gestational length. In summary, these aberrations may contribute to an increased risk for islet autoimmunity and subsequent development of type 1 diabetes in the offspring.
Supported by: an EFSD Clinical Research Grant

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First trimester serum cytokine levels and the development of autoimmune disease in offspring

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Background and aims: Pregnancy involves local and systemic changes in the balance between the Th1 and Th2 immunological response. It is generally accepted that pregnancy is mediated by a Th2 response inducing tolerance in the mother towards the fetal allograft. Several studies suggest that a shift in the Th1/Th2 balance during pregnancy caused by underlying environmental factors could be associated with post-partum autoimmune disease in the offspring. In this study we used Celiac disease as a model to investigate if autoimmunity is triggered already *in utero* during early pregnancy, observed as changes in the mother's cytokine profile.
Material and methods: Ten cytokines were measured by electro chemi-luminiscent multiplex ELISA in serum samples obtained from mothers during the first trimester. Cases included women with children who before the

age of 5 developed verified Celiac disease. Matched controls were selected based on age, Celiac Disease associated HLA genotype and serum sampling date. Mann-Whitney U tests first tested for a significant overall shift in cytokine levels in cases compared to controls. Chi-square tests further examined whether the cases were distributed evenly across quartiles ranges of the control distribution.
Results: We observed that seven out of ten cytokines were significantly increased in the cases when compared to matched controls. Five of the cytokines were Th1 mediated (TNFα, IFNγ, IL-2, IL-1β, IL-12), and two were Th2 mediated cytokines (IL-13 and IL-10). In the matched case-control analysis, the three top cytokines were shown to be: TNFα (p=0.002), IL-13 (p=0.002) and IFNγ (p=0.005) which all were all elevated in the case group.
Conclusion: A delicate balance between Th1/and Th2 mediated cytokines is required to ensure a successful pregnancy. However, changes in this balance could predispose the fetus to future disease. In this study we show that autoimmunity in children is triggered already during early pregnancy and can be observed as quantitative changes in the serum cytokine levels of pregnant mothers.
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Maternal serum 25-hydroxy-vitamin D during late pregnancy and risk of type 1 diabetes in the offspring
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Background and aims: A few case-control studies and one cohort study have suggested that use of vitamin D supplement in childhood or by the mother during pregnancy may be associated with lower risk of type 1 diabetes in children. However, vitamin D status is influenced not only by dietary intake (from food and supplements) but also skin exposure to ultraviolet light. No published study has yet reported the possible relation between the serum level of 25-hydroxy-vitamin D, which is a good marker of the integrated effects of dietary and endogenous sources of vitamin D, and the risk of type 1 diabetes. We aimed to test whether higher maternal serum concentration of 25-hydroxy-vitamin D during late pregnancy predicts a lower risk of childhood onset type 1 diabetes in the offspring.
Materials and methods: Based on a prospective cohort of nearly 30 000 pregnant women who gave birth in Norway during 1992-94, we analysed serum samples from 99 pregnant women whose child developed type 1 diabetes before 15 years of age and 155 randomly selected control women whose child did not develop type 1 diabetes during follow-up. The sera were collected around week 37 of pregnancy and stored at -20°C until analysed in 2008/9. Cases were identified by record linkage to The Norwegian Childhood Diabetes Registry. Serum 25-hydroxy-vitamin D was analysed using a radio immunoassay (DiaSorin). Power calculations showed 88% power to detect a significant association with a test for trend over quartiles with 100 cases and 150 controls, assuming an odds ratio of 0.33 comparing the upper vs. lower quartile of 25-hydroxy-vitamin D and a logit-linear dose-response relation.
Results: There were no significant differences between cases and controls in demographic data. The mean level of 25-hydroxy-vitamin D in cases was 66.4 nmol/l and in controls 71.5 nmol/l, odds ratio (per nmol/l): 0.9 (95% CI 0.9-1.0, p=0.1). Comparing the 4th vs. 1st quartile of maternal serum 25-hydroxy-vitamin D, the odds ratio was 0.6 (95% CI 0.3-1.3). Test for trend over quartiles: p=0.2. Adjustment for season of blood collection, maternal diabetes or other potential confounding variables did not influence these results.
Conclusion: In this first study to test the hypothesis that high serum 25-hydroxy-vitamin D status during pregnancy predicts a lower risk of type 1 diabetes in children we found no statistically significant association despite a suggestive trend.
Supported by: South–Eastern Norway Regional Health Authority

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No association of human enterovirus RNA in monthly faecal samples and islet autoimmuinity in the Norwegian MIDIA study
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Background and aims: To test whether the frequency of human enterovirus RNA in faecal samples collected monthly from early infancy was associated with development of multiple islet autoantibodies in children with the highest risk HLA genotype.
Materials and methods: Individuals carrying the HLA-DRB1*0401-DQA1*03-DQB1*0302/DRB1*03-DQA1*05-DQB1*02 genotype were identified at birth and followed with monthly stool samples from 3 to 35 months. Blood samples taken at age 3, 6, 9, 12 months, and then annually, were tested for autoantibodies to insulin, glutamic acid decarboxylase 65 and protein tyrosine phosphatase IA-2. Twenty seven children developed positivity for at least 2 islet autoantibodies in 2 or more consecutive samples (cases). Two matched controls per case were selected. Stool samples from these children were analyzed for enterovirus with a semiquantitative real-time reverse transcriptase PCR. The frequency of enterovirus was modelled as the dependent variable and took account of the intra-individual correlation in enterovirus infection using a random intercept for the enterovirus infection. The data was also analysed using conditional logistic regression modelling with islet autoimmunity as the outcome.
Results: The frequency of enterovirus RNA in stool samples from cases prior to seroconversion (43/339, 12.7%) did not differ from the frequency in matched controls (94/692, 13.6%); odds ratio=1.01 (95% CI: 0.59 - 1.72), P=0.97. Results remained essentially unchanged after adjustment for potential confounders, restriction to various time windows before seroconversion, infections in the first year of life, or after including samples collected after seroconversion. There was no difference in the average quantity of enterovirus RNA, or the frequency of repeatedly positive samples. In the conditional logistic regression analysis, the "odds ratio" per enterovirus infection was 1.12, with corresponding 95% confidence interval 0.66-1.91.
Conclusion: The data strongly suggest that faecal shedding of enteroviral RNA does not predict islet autoimmunity, as human enterovirus infections are not more frequent before or after autoantibodies appear.

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Identification of type 1 diabetes-associated methylation variable positions that precede disease diagnosis
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Background and aims: Type 1 diabetes (T1DM) is a complex multifactorial autoimmune disease caused by a combination of genetic and non-genetic factors. A role for the latter is suggested by studies of migrant populations, twin-cohorts, and the recent rise in T1DM prevalence. To assess whether epigenetic factors could contribute to these non-genetically determined effects we performed a genome-wide, promoter-specific DNA methylation analysis.
Materials and methods: We studied $CD14^+$ monocytes from 15 childhood-onset T1DM-discordant monozygotic (MZ) twin pairs, 9 control MZ twin pairs, plus 7 non-diabetic antibody-positive children studied prospectively before and after they developed T1DM. Methylation profiling was done using Illumina HumanMethylation27 BeadChips which allow for DNA methylation analysis of >27,000 CpG sites associated with >14,000 promoters per sample.
Results: We identified 132 T1D-associated methylation variable positions (T1D-MVPs) (P=0.02). Importantly, T1D-MVPs displayed statistically sig-

nificant trends for methylation differences in the expected direction in an independent set of T1DM singletons and controls, both before (P=0.001) and after (P=0.015) disease-onset, indicating epigenetic variation before T1DM clinical onset.
Conclusion: The identified T1DM-MVPs are associated with genes involved in pathways strongly implicated in the aetiopathogenesis of T1DM including major antigen expression, proinflammation, regulation of immunoglobulin secretion and apoptosis, with only a small over-representation of T1D-MVPs within known T1DM genetic susceptibility regions. Changes in DNA methylation in critical immune-response pathways probably contribute to the pathogenesis of T1DM.
Supported by: JDRFI

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A lipotoxicity model in the INS-1 832/13 beta cell line and the epigenetic alterations induced by it
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Background and aims: The hallmarks of Type 2 Diabetes Mellitus (T2D) are peripheral insulin resistance that in combination with impaired insulin secretion results in hyperglucemia. While T2D has a strong genetic component, the disease can be triggered by obesity and a sedimentary lifestyle. Plasma free fatty acids (FFA) are elevated in obese subjects and this is believed to be an important pathogenic factor in T2D. Histone proteins and the nucleosomes they form with DNA are the fundamental building blocks of chromatin. Histone acetylation is a chromatin modification associated with an open chromatin structure and increased gene transcription. Histone acetyl transferases (HATs) and histone deacetyltransferases (HDACs) are enzymes which regulate histone acetylation. It has previously been demonstrated that the regulation of insulin expression by glucose is under the control of histone acetylation. The aim of our study is to examine whether a lipotoxic challenge of clonal 832/13 beta-cells induces epigenetic alterations and impaired metabolism.
Materials and methods: Insulin secretion for one hour in static incubation was measured by RIA. Differences in glucose metabolism were assessed by Extracellular flux analyzer XF24 (Seahorse Bioscience, Billerica, MA). HAT and HDAC activity was measured using a Nuclear/Cytosol Fractionation Kit (BioVision, Mountain View, CA) and HAT/HDAC colorometric assays (BioVision, Mountain View, CA).
Results: Lipotoxic conditions, assessed as 0,5 mM palmitate for 48 h, significantly increased basal secretion at 2,8 mM of glucose from 9 ±3 ng/mg/h to 29 ±6 ng/mg/h (p<0,001) and significantly decreased glucose-stimulated insulin secretion at 16,7 mM of glucose in beta-cells from 172 ±60 ng/mg/h to 52 ±15 ng/mg/h (p<0,05). In extracellular flux (XF) measurements lipotoxic beta-cells failed to increase oxygen consumption rates (OCR) in response to elevated glucose to the same extent as control cells. While 16,7 mM of Glucose increased OCR expressed as area under curve (AUC) with 32 ±0,02 % the same increase was only 12 ±0,03 % under lipotoxic conditions (p<0,05). This suggests that the lipotoxic beta-cells have a diminished ability to increase ATP production in response to increases in glucose concentration. HAT activity was elevated more than 4-fold in lipotoxic cells versus control cells (p<0.05), which suggests that elevated levels of FFA may induce epigenetic changes and gene transcription in the clonal 832/13 beta-cells. HDAC activity was not affected by lipotoxicity.
Conclusion: The lipotoxicity model we established affected glucose-stimulated insulin secretion in a way which mimics that seen in T2D patients. We can also conclude that part of this is due to metabolic effects such as lack of increased OCR, probably reflecting a decrease in ATP production. Finally, elevations in HAT activity indicate that lipotoxicity induces activation of genes in the clonal 832/13 beta-cells.

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Smoking impairs glucose control in patients with type 1 diabetes mellitus: a prospective, longitudinal single-center study
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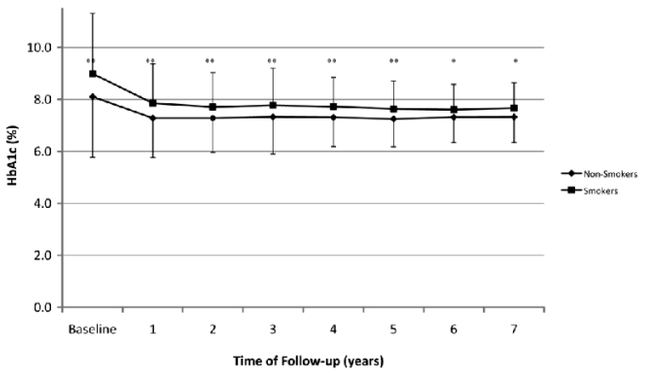
Background and aims: Smoking is known to negatively influence metabolic control in patients with type 1 diabetes mellitus. However, previous multicenter cross-sectional studies lack information on possible differences in diabetes therapy and consultation adherence between smokers and non-smokers. The aim of this prospective single-center study was to determine the effect of smoking on metabolic control during a longitudinal observation period.
Materials and methods: Patients with type 1 diabetes mellitus who were referred to our institution were included if written informed consent was given. Data on smoking habits and metabolic control (HbA_{1c}) were taken at baseline and during follow-up, as well as on insulin dosage, weight, blood pressure and serum lipids. All patients were seen every 3 to 4 month and treated with intensive insulin therapy or continuous subcutaneous insulin infusion.
Results: 763 patients were included, 160 (17.1%) were smokers. HbA_{1c} levels differed significantly between current smokers and non-smokers at baseline and during follow-up (mean 6.6 years, mean HbA_{1c} 7.9±1.3 vs 7.3±1.1%, p=0.001) (figure 1, **p<0.001, *p<0.05). There was no difference between smokers and non-smokers in terms of age (35.1±12.8 vs 36.1±14.2y), diabetes duration (11.5±10.8 vs 13.4±12.5y) and BMI (23.6±4.5 vs 23.9±5.8kg/m^2). However, there was a significant difference in gender between the two groups (71.3% male in smokers, 52.1% in non smokers). Therefore, every analysis comparing the two groups was adjusted for gender. At the end of follow-up, weight, blood pressure and serum lipids were not different between the two groups when adjusted for gender. The proportion of patients with two or more diabetes related complications was higher in smokers at the end of follow-up (p=0.04). Insulin requirement at the end of follow-up was higher in smokers than in non-smokers (0.71±0.30 vs 0.65±0.31IU/kg/d, p=0.04), whereas there was no difference in the occurrence of severe hypoglycemias (16 events per 100 patient years in smokers, 17 in non-smokers).
Conclusion: In conclusion, this study demonstrates that patients with type 1 diabetes mellitus who smoke have a significantly worse metabolic control than non-smokers despite the same quality and intensity of diabetes treatment.

Figure 1



PS 11 Ethnic differences in metabolic traits

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A comparison of diabetes incidence in Whites, South Asians, Chinese and Blacks: a population-based cohort study in Ontario, Canada

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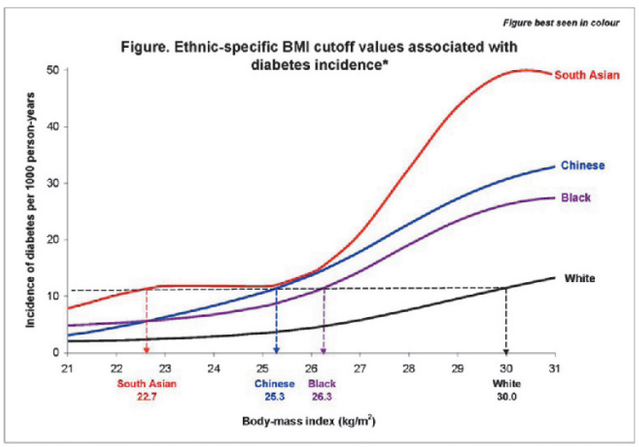
Background and aims: Diabetes is a growing epidemic in many countries worldwide. While ethnic differences in the prevalence of diabetes is well documented, little is known about the relative *incidence* of diabetes across the world's four major racial-ethnic groups; Whites, Chinese, South Asians, and Blacks. We therefore conducted a population-based comparison of incidence rates of diabetes across Whites, Chinese, South Asians, and Blacks living in Ontario, Canada. We also derived ethnic-specific body-mass index (BMI) cutoff values to define obesity based on clinically-ascertained diabetes.
Materials and methods: We conducted a cohort study of 59824 non-diabetic adults (57210 Whites, 866 Chinese, 1001 South Asians, and 747 Blacks) aged 30 years or older, who were derived from Statistics Canada's population health surveys (1996-2005). Subjects were followed up for up to 12 years for diabetes incidence using record linkages to the Ontario Diabetes Database, an administrative-based algorithm shown to identify diabetes with 86% sensitivity and 97% specificity.
Findings: Diabetes incidence (per 1000 person-years) was highest among South Asians (20.8), followed by Blacks (16.3), Whites (9.5), and Chinese (9.3). Cox proportional hazards regression models adjusted for age, sex, BMI, and sociodemographic characteristics revealed hazard ratios (HR) that were significantly higher in South Asians (HR: 3.40), Blacks (HR: 1.99), and Chinese (HR: 1.87) than Whites (all p<0.0001). The median age at diagnosis was 9 years younger in South Asians and 3 years younger in Chinese than in Whites. Ethnic-specific BMI cutoff points for diabetes risk were identified using Poisson regression and restricted cubic splines. For the equivalent incidence rate of diabetes at BMI 30 kg/m^2 in Whites, the candidate BMI cut-off points were 23 kg/m^2, 25 kg/m^2, and 26 kg/m^2 for South Asians, Chinese, and Blacks, respectively (see Figure).
Interpretation: Current screening and prevention strategies for diabetes are informed mainly by studies of White populations; however, our study suggests that the risk of new diabetes is significantly greater in South Asians, Chinese, and Blacks; that these groups present with diabetes at younger ages; and that the current definition of obesity is inadequate for assessing diabetes risk in these non-White groups. Ethnic-specific prevention programs and equitable health services are needed to reduce the burden of diabetes in these high-risk populations.



*Association between body-mass index (BMI) and incidence of diabetes (per 1000 person-years), by racial-ethnic group, Ontario, Canada. Poisson regression and restricted cubic splines were applied; models were adjusted for age, sex, race-ethnicity, BMI, BMI-ethnicity interaction, age-BMI, income adequacy, survey year, and urban vs. rural dwelling.

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The association between physical activity and type 2 diabetes according to weight status among different ethnic groups

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Background and aims: Physical inactivity and adiposity are both independently related to type 2 diabetes (DM). Some studies have suggested that a high level of physical activity can counterbalance the negative health effects of obesity. This implies a differential effect of physical activity according to weight status. Moreover, effects of physical activity may potentially differ between ethnic groups. Therefore, we studied differences in the association between physical inactivity and DM according to weight status among individuals from different ethnic groups.
Materials and methods: We analysed data on 508 White Dutch, 596 African-Surinamese and 339 Hindustani Surinamese participants, aged 35-60 years, in the population-based, cross-sectional SUNSET study. Physical activity was measured using the Short Questionnaire to Assess Health-enhancing Physical Activity, which covers similar topics as the long-format International Physical Activity Questionnaire and has been validated for the Dutch population. Physical inactivity was defined as the lowest quartile of reported activity (min/week). Overweight was primarily defined as a BMI > 25 kg/m^2, and in a second analysis as a waist circumference ≥ 94 cm in men and ≥ 80 cm in women. DM was defined based on fasting plasma glucose levels and self-reported diagnosis of DM.
Results: Physical inactivity was independently associated with DM; after adjustment for sex, age, ethnicity and BMI, the odds of having diabetes was 1.69 (95% CI: 1.08-2.63) higher in individuals in the lowest quartile of physical activity, compared to those in the highest quartile. This association was present in both overweight individuals and those with a normal BMI, although this was only significant in overweight individuals (normal BMI: OR 1.51, 95% CI 0.60-3.75, overweight: OR 1.79, 95% CI 1.07-2.98). The association between physical inactivity and diabetes was stronger in ethnic Dutch, (OR 4.81, 95% CI 1.32-17.61) than in Hindustani Surinamese (OR 1.50, 95% CI 0.76-2.98) and African Surinamese (OR 1.40, 95% CI 0.70-2.81), after adjustment for sex, age and BMI. As also observed in the total population, weight status did not alter the association between physical activity and DM within the ethnic groups. Similar results were obtained when using waist circumference as an indicator of overweight.
Conclusion: Physical inactivity was independently associated with type 2 diabetes among both individuals with overweight and individuals with normal weight. This confirms the importance of regular exercise for all. However, the results suggest that potential health gain may differ between ethnic groups.

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Risk factors associated with age at diagnosis of type 2 diabetes mellitus in a bi-ethnic population

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Background and aims: Ethnic minorities have higher prevalence of type-2 diabetes mellitus (T2DM) as compared to majority population groups. Younger age at diagnosis may further increase the burden of disease in ethnic minority groups. We explored factors associated with age at diagnosis of T2DM in the Arab minority and the Jewish majority groups in Israel.
Materials and methods: Participants (1,100; age: 25-74 years) were selected at random from the urban general population in the Hadera district in Israel. Information collected by interviews included socioeconomic status (SES) parameters, diabetes status, lifestyle habits (including dietary intake till T2DM diagnosis or interview), height, body weight during most of adult life, and history of diabetes in first degree relatives. Family history score of diabetes (T2DM FHScore) and dietary energy density (DED) in calories/grams were calculated. Factors associated with age at diagnosis of T2DM were tested using a multivariate Cox proportional hazard model.
Results: Of 1,093 participants with information on diabetes status, 180 had T2DM (16.5%). Arabs had higher prevalence of T2DM than Jews (21.0% vs. 12.0%; HR: 1.99 [95%CI: 1.47-2.71], P<0.01). The mean (SD) age at T2DM diagnosis was 52.2 (10.2) years in Arabs vs. 56.1 (10.8) in Jews; p=0.02. By the age of 57 years, 25% of the Arab participants had T2DM. The corresponding

age in the Jewish participants was 68 years (p<0.0001). In multivariate analysis, Arabs had 1.70 times greater risk for T2DM compared to Jews (95%CI: 1.19-2.43), adjusted for gender, BMI during most of adult life, T2DM FHScore and DED. Other factors associated with risk of T2DM included: higher BMI during most of adult life, higher T2DM FHScore, and higher DED (see table). SES parameters, cigarette smoking, gender, and current physical activity were not significantly associated with the risk for T2DM.
Conclusion: Compared to the Jewish majority group, people of the Arab minority group in Israel are at higher risk for having T2DM at a younger age, and greater loss of healthy life-years. Efforts to prevent or delay the onset of T2DM should be directed towards ethnic minority groups at high risk, in order to reduce diabetes-related health disparities.

Table: Factors associated with the risk for T2DM

Risk Factor*	Hazard Ratio (HR); 95% confidence interval (CI)
Ethnic group (Arabs vs. Jews)	1.70; 1.19-2.43
BMI during most of adult life (highest vs. tertile)	2.07; 1.34-3.21
T2DM FHScore (highest vs. lowest)	5.78; 3.99-8.36
DED (highest vs. lowest quartile)	1.67; 1.08-2.61

*-adjusted for gender

Supported by: The Israel National Institute for Health Policy & Health Services Research

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Ethnic-specific cut-points for central obesity measures for predicting insulin resistance in South African women

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Background and aims: The relationship between waist circumference (WC), visceral adipose tissue (VAT) and insulin resistance (IR) differs by ethnicity. The aim of the present study was to explore ethnic-specific cut-points for measures of central obesity for predicting IR as estimated by the homeostasis model (HOMA-IR) in black and white women, and to determine the optimal measure of central obesity i.e. WC vs. waist-height-ratio (WHtR) vs. VAT that best predicts IR in each ethnic group.
Materials and methods: Anthropometry (weight, height, WC, hip circumference), VAT (computed tomography) and HOMA-IR were measured in 241 black and 188 white premenopausal non-diabetic South African women, free from known disease and not on medication. IR was defined as the upper tertile of HOMA-IR for the whole group. The Youden index was calculated to determine the 'optimal' cut-point for WC, WHtR and VAT that best predicted IR. The accuracy of each measure to predict IR was assessed using receiver operator characteristic (ROC) curves.
Results: Ethnic-specific cut-points for central obesity measures for predicting IR are presented in Table 1.
Conclusion: In our population of apparently healthy black and white South African women, we show that measures of central obesity better predict IR in white than black women, but there is little difference between WC, WHtR and VAT in their ability to predict IR in both ethnicities. As we show that VAT adds no advantage in the prediction of risk, WC or WHtR, basic cost-effective anthropometrical measures, should be used to identify risk. Long-term prospective studies are required to examine whether individuals from these ethnic groups who exceed these cut-points develop cardiovascular disease and diabetes and/or whether these relationships are similar in groups with known disease or pathophysiology.

Table 1: Optimal WC, WHtR and VAT cut-points which best predict IR defined as HOMA-IR >2.09

	Black Women			White Women		
	ROC AUC ± SE	Cut-point	J value	ROC AUC ± SE	Cut-point	J value
WC (cm)	0.76 ± 0.03	>94	0.42	0.85 ± 0.03	>96	0.60
WHtR	0.77 ± 0.03	>0.62	0.44	0.84 ± 0.03	>0.59	0.55
VAT (cm2)	0.72 ± 0.04	>78	0.36	0.81 ± 0.04	>100	0.47

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The impact of migration on metabolic outcomes and coronary heart disease risk: a comparison of migrant South Asians, Asian Indians and white Europeans

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Background and aims: Given the consistent findings of increased prevalence, premature onset and increased mortality from CHD in South Asian (SA) individuals, there is a need to determine the underlying causes in order to develop effective prevention and treatment strategies. One fifth of the developing world is represented by individuals of a SA origin and migration has resulted in large numbers of SAs settling in many developed countries. We investigated the impact of migration upon metabolic and CHD risk factors.
Materials and methods: This cohort consisted of 2287 White Europeans (WEs), 1007 SAs living in India (ISA) and 927 Migrant SAs (MSA) residing in the United Kingdom (UK). All subjects were aged 40-75 years. The WE and MSA cohort were recruited from a cross-sectional diabetes screening study conducted in Leicestershire, UK. ISA participants were recruited from a community-based epidemiological study, undertaken in Punjab, India. All participants underwent metabolic and anthropometric measurements. All those with established or newly diagnosed diabetes and CHD were excluded from analysis. Data is presented as mean ± SD or number (%). The MSA group were used as reference. P values were calculated using between groups ANOVA (*p<0.05, **p<0.001; see table 1). Non-parametric data were analysed using the Kruskal-Wallis test. χ^2 test was used for categorical data.
Results: MSAs had a significantly higher mean BMI and waist circumference for both males and females (p< 0.001) compared to ISAs; however, WEs had a significantly larger waist circumference than both Asian groups (see table 1). These relationships remained statistically significant after adjustment for age. MSAs had a significantly higher fasting plasma glucose compared to ISAs (p<0.05); furthermore, the prevalence of the metabolic syndrome (International Diabetes Federation criteria) was greater in MSAs compared to both ISAs (p<0.001) and WEs (p<0.05). MSA males had significantly higher cholesterol compared to ISAs (5.1mmol/L vs. 4.8mmol/L, p<0.05). In addition, MSA males had a significantly higher 10-year CHD risk (Framingham) compared to WEs (p<0.05); however, WE and ISA females had a significantly higher risk than MSAs (p<0.05).
Conclusion: This large dataset demonstrates that factors associated with migration serve to exacerbate some important indicators of cardiovascular and diabetes risk such as BMI, fasting glucose, serum cholesterol and waist circumference; however, certain modifiable risk factors, such as smoking were higher in ISAs females compared to MSAs, which contributed to a higher CHD risk in this group.

Table 1. Comparison of lifestyle and anthropometric variables by ethnicity and sex

	Females			Males		
	Migrant South Asian	Asian Indian	White European	Migrant South Asian	Asian Indian	White European
BMI (kg/m^2)	27.9 ± 5.1	23.5 ± 5.9**	28.3 ± 5.8	26.1 ± 3.4	23.4 ± 5.2**	27.9 ± 4.4**
Waist circumference (cm)	90.4 ± 11.3	82.4 ± 13.8**	92.0 ± 13.7*	95.5 ± 9.9	90.8 ± 15.3**	99.3 ± 12.0**
Fasting plasma glucose (mmol/L)	5.1 ± 1.1	4.9 ± 2.0*	5.0 ± 0.8	5.3 ± 0.6	4.9 ± 0.8**	5.2 ± 1.1
Hypertension	89 (21)	213 (55.5)**	460 (37.7)**	162 (36.7)	136 (49.1)*	434 (45.1)*
Smoker	3 (0.7)	107 (22.7)**	152 (28.7)**	70 (15.8)	101 (36.1)**	280 (28.9)**
Total cholesterol (mmol/L)	5.2 ± 0.9	5.2 ± 1.2	5.6 ± 1.0**	5.1 ± 0.9	4.8 ± 1.0*	5.4 ± 1.0*
Metabolic syndrome (International Diabetes Federation criteria)	355 (83.9)	291 (58.9)**	994 (81.2)*	315 (71.4)	169 (52.5)**	663 (68.6)*
Framingham Coronary Heart Disease risk (10 year)	4.8 ± 3.9%	6.5 ± 6.2%*	7.5 ± 4.7%*	18.3 ± 12.2%	18.5 ±13.6%	15.6 ± 7.6%*

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Levels of 25-OH-vitamin D in early pregnancy in women from five ethnic groups with and without gestational diabetes

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Background: The STORK Groruddalen Research Program was set up to identify predictors for gestational diabetes (GDM) and foetal growth in a multiethnic population in Oslo. Inclusion will finish in May 2010. Poor vitamin D status has been linked to insulin resistance and in some studies it has been associated with GDM.
Aims: To assess levels of 25-OH-vit. D in early pregnancy in women from five ethnic groups who later developed GDM compared to women without GDM.
Methods: This is a population-based cohort study of pregnant women attending the Child Health Clinics in Groruddalen and their offspring. Information and questionnaires were translated to eight languages, covering the largest ethnic groups. Women were eligible if 1) living in the districts, 2) planned giving birth at the study hospitals, 3) in gestational week (GW) ≤20, 4) not suffering from diseases necessitating intensive hospital follow-up during pregnancy, 5) could communicate in Norwegian or any of the translated languages and 6) able to give informed consent. Ethnic origin in the present study: Europe (including North America), South Asia, East Asia, Middle East (including North Africa/Central Asia) and Somalia. Questionnaire data, blood pressure, anthropometric measurements, fasting blood and urine samples collected by midwifes were obtained at GW 10-20, 28 and 12 weeks postpartum. A 75 g OGTT was performed at GW 28 (24-32), glucose analyzed on site in venous EDTA blood samples (HemoCue, Angelholm, calibrated for plasma). The diagnosis of GDM was based on the WHO-criteria: fasting ≥7.0 or 2-hour value ≥7.8 mmol/l. 25-OH-vitamin D was measured by a radioimmunoassay method (DiaSorin, Stillwater, MN, USA). Descriptive analyses, ANOVA for continuous variables, chi-square tests for categorical variables and logistic regression analyses were performed.
Results: By March 1st 2010, 744 women were included (From Europe: 81.2% of the invited, Asia: 66.0%, Middle East: 64.5%, Africa: 60.3%). OGTT data were available from 539 women and 78 GDM cases (14.5%) were identified The crude prevalence of GDM was high in all groups (Europe: 12.7%, South Asia 13.4%, East Asia 24,0%, Middle East 22.4%, Somalia 18.5%)(Table 1). East Asian GDM women had a lower mean BMI than European GDM women ($p<0.05$). In women without GDM mean BMI was higher in the Middle East group than in Europeans ($p<0.05$). Odds ratio for GDM adjusted for age, parity, BMI and GW for minority groups from Asia and Africa compared to Europeans was 1.5-2.7, borderline significant for women from Middle East ($p=0.057$) and East Asia ($p=0.054$). 25-OH-vit. D was significantly lower in both GDM and non-GDM women from ethnic minorities compared to Norwegians, but no significant differences between GDM and non-GDM women were found.
Conclusion: The crude prevalence of GDM was high in all groups, but highest in groups from Asia and Africa. Low levels of 25-OH-vit.D was significantly associated with ethnicity, but not with GDM.

	Europe		Middle East		South Asia		East Asia		Somalia	
	GDM	Non GDM	GDM	Non GDM	GDM	Non GDM	GDM	Non GDM	GDM	Non GDM
N % GDM	34 12.7	234	15 22.4	52	18 13.4	114	6 24.0	19	5 18.5	22
Age years (mean)	31.8	30.6	33.7	29.2	31.4	28.4	33.1	30.5	35.8	27.4
Parity	0.6	0.7	1.7	1.1	1.6	1.2	0.3	1.0	3.0	2.0
Gest. week (GW) at inclusion	14.3	14.6	16.4	15.8	17.1	15.3	16.1	16.5	20.3	17.9
BMI kg/m^2	27.3	24.5	26.7	27.6	26.2	24.1	22.1	23.0	30.6	25.4
25-OH-vitamin D (nmol/l)	66.9	69.6	44.9	33.8	41.3	31.9	50.2	49.5	26.8	36.6
25-OH-vitamin D adj. for age, GW	68.3	69.1	43.7	32.2	41.6	32.2	49.6	48.7	21.9	35.9

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Trends in the prevalence of diabetes based on the level of education among Korean women: Korea National Health and Nutrition Examination Survey 1998-2007

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Objective: This study was designed to determine the differences in the prevalence of diabetes based on the level of education in Korean women.
Research design and methods: A total of 3,842, 2,894, 3,020, and 1,647 women were included from The Korea National Health and Nutrition Examination Survey 1998, 2001, 2005, and 2007 surveys, respectively. Diabetes was defined as a self-reported history or a fasting plasma glucose ≥126 mg/dl. Education was classified into the following three categories: low (less than high school graduate), medium (less than college graduate), and high (college graduate or more). A linear model was used to test a trend of diabetes prevalence according to educational health inequality. A multiple logistic regression analysis was performed to estimate odds ratios for diabetes prevalence according to education levels.
Results: The prevalence of diabetes among Korean women was decreased from 8.1% in 1998 to 6.6% in 2007. Between these periods, there was a significant decreasing trend of the prevalence from 2.8% to 0.7% among women with a high education level, but no significant change was observed, such as from 12.2% to 15.3% among those with a low level of education ($p=0.002$ and $p=0.15$, respectively). A difference of diabetes prevalence between the high and low levels of education increased significantly across survey periods ($p=0.0007$). The odds ratio for the prevalence of diabetes in women with a low level of education compared to women with a high level of education increased from 1.6 in 1998 to 2.2 in 2001, 3.4 in 2005, and 4.8 in 2005 after adjusting for age, BMI, smoking, and exercise.
Conclusion: The prevalence of diabetes had an inverse relationship with the level of education in Korean women during last decade. More than the negative relationship, an educational health inequality for the prevalence of diabetes was widened during Korea's rapid economic development. Further studies on the causes of educational inequalities in the prevalence of diabetes, as well as the development of an effective intervention program, are imperative to reduce the prevalence of diabetes and a gap of the prevalence in Korean women.

PS 12 Environmental factors and type 2 diabetes mellitus

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Dairy consumption and insulin resistance syndrome: results from a French prospective study, D.E.S.I.R., data from the Epidemiological Study on the Insulin Resistance syndrome

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Background and aims: In the French population from the D.E.S.I.R. cohort, cross-sectional analyses have shown that a higher consumption of dairy products or calcium is associated with a lower prevalence of the insulin resistance syndrome (IRS). The aim of our study was to assess the influence of dairy products on the nine year cumulative incidence of the IRS or associated diseases in this population-based prospective study with a 9-year follow-up, D.E.S.I.R..
Materials and methods: In total, 5212 volunteers from the western central part of France were included in the cohort. A questionnaire was completed by each participant at baseline, to determine the frequency and level of consumption of different foods. Two items concerned dairy products (cheese, milk and other dairy products). There were 4 groups according to the intake of dairy products (except cheese) and 3 groups for cheese intake. Calcium intake was calculated from the questionnaire. Calcium density of the diet was defined as the amount of calcium ingested per 1000kCal. Sex-specific calcium density quartiles were calculated. The associations between these dairy variables at inclusion and the incidence of metabolic diseases were tested using logistic regression models adjusted 1) for sex, age, alcohol, smoking, physical activity, fat intake and 2) the same covariates plus BMI. The odds ratios were determined by the logistic regression, indicating the risk for a change from one group to the next, e.g. from one quartile of calcium density to the next quartile. The association of dairy products with continuous variables was tested by analysis of covariance for repeated measures, using the same covariates as for the logistic regression.
Results: The consumption of dairy products other than cheese and calcium density of the diet were inversely associated with the incidence of the IRS and with the incidence of impaired fasting glycaemia (IFG) or type 2 diabetes (T2D) during the 9-year follow-up. The consumption of cheese was negatively associated with the incidence of the IRS, in particular after adjustment for BMI. (Table). These 3 parameters were associated with lower 9-year means of diastolic blood pressure, plasma triglycerides and insulin levels and with lower BMI gain in this period. Higher cheese intake and calcium density were associated with lower increase in waist circumference and plasma triglyceride levels. Dairy products and cheese intake were associated with a lower increase in blood pressure during the follow-up.
Conclusion: A higher consumption of dairy products and calcium reduces the incidence of the IRS during a 9-year period in a large cohort drawn from the French general population. This inverse association is observed with most of the traits of the metabolic syndrome. These results indicate that dairy product consumption could improve the cardiovascular risk.

Odds ratios (95% CI) of developping a metabolic disease during the 9-year follow-up

	IRS (IDF definition)		IRS (NCEP definition)		IFG + T2D	
Dairy products (except cheese)						
Model 1	0.89 (0.80-0.99)	*p=0.03*	0.87 (0.78-0.98)	*p=0.02*	0.85 (0.76-0.95)	*p=0.005*
Model 2	0.91 (0.81-1.00)	*p=0.07*	0.91 (0.81-1.03)	*p=0.13*	0.86 (0.77-0.97)	*p=0.01*
Cheese						
Model 1	0.88 (0.75-1.03)	*p=0.11*	0.78 (0.65-0.93)	*p=0.01*	0.87 (0.73-1.04)	*p=0.14*
Model 2	0.82 (0.69-0.97)	*p=0.02*	0.70 (0.58-0.86)	*p=0.004*	0.84 (0.70-1.00)	*p=0.06*
Calcium density						
Model 1	0.96 (0.89-1.03)	*p=0.28*	0.92 (0.85-1.00)	*p=0.06*	0.93 (0.85-1.01)	*p=0.08*
Model 2	0.91 (0.84-0.99)	*p=0.03*	0.88 (0.81-0.97)	*p=0.008*	0.91 (0.84-0.99)	*p=0.03*

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High intake of fermented milk is associated with decreased risk of type 2 diabetes and better insulin sensitivity

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Background and aims: Several observational studies have shown an inverse association between intake of dairy products and risk of type 2 diabetes (T2D). Fermented dairy products contain probiotic bacteria that may influence the composition of the gut microbiota, which are suggested to play a crucial role in the development of metabolic disorders; however, studies examining the association with fermented milk are lacking. We therefore explored the association between intake of specific dairy products (fermented milk, non-fermented milk, cheese (>10% fat), cream and butter) and incident T2D using the large Swedish Malmö Diet and Cancer study (MDCS) with comprehensive and detailed data on dairy food intakes. A genetic variant near the insulin receptor substrate 1 gene (*IRS1*) has been shown to associate with insulin resistance and T2D and we therefore wanted to examine if the associations between dairy intake and T2D differentiate depending on *IRS1* genotype.
Materials and methods: Among participants in the MDCS without a history of cardiovascular disease and diabetes (n=26,369; 44-74 y of age; 62% females), 1,616 individuals with incident T2D were identified from national and regional registers during a mean period of 12 y follow-up. Fasting blood glucose and plasma insulin were measured in 4,628 of the subjects at baseline, and HOMA index was used as a measure of insulin resistance. A total of 24,132 of the individuals were genotyped for the genetic variant in *IRS1* (rs2943641). Dietary data was collected using a modified diet history method. Cox proportional hazard regression was used to calculate hazard ratios (HR) for each energy-adjusted food group adjusted for several potential confounders (i.e. age, sex, energy intake, BMI, smoking habits, alcohol consumption, leisure-time physical activity, and education). In sensitivity analyses we excluded individuals reporting dietary change in the past as they are suspected to have unstable food habits. The interaction between *IRS1* genotype and food variables on incident T2D was assessed by introducing a multiplicative factor with continuous variables in the multivariate analyses.
Results: After adjusting for potential confounders, high intake of fermented milk was associated with lower HOMA index (*P*-trend=0.002), whereas high intakes of non-fermented milk and butter were associated with higher HOMA index (*P*-trend=0.0002 and 0.006, respectively). Without taking dietary change in the past into account, high intakes of cream, cheese and butter were associated with decreased risk of T2D (P-trend<0.05 for all). However, after excluding those 22% individuals reporting dietary change in the past, only high intake of fermented milk was significantly associated with decreased incidence of T2D (HR, 0.76; 95% CI, 0.64-0.91 for highest tertile of consumers vs. zero-consumers; *P*-trend=0.008). The C allele of rs2943641 was associated with insulin resistance assessed by the HOMA index (P=0.04) and T2D risk (OR, 1.13; 95% CI, 1.04-1.21). However, we observed no significant interaction between *IRS1* genotype and food variables on incident T2D.
Conclusion: This study indicates that intake of fermented milk is associated with better insulin sensitivity and that a high intake may reduce the risk of T2D. Our observation suggests that it is crucial to separate the effect of fermented and non-fermented milk products when investigating the health effect of dairy foods.
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Alcohol consumption and the risk of developing prediabetes and type 2 diabetes in Swedish middle-aged men and women

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Background and aims: Type 2 diabetes (T2D) has become a major health problem in many industrialized countries. Alcohol consumption represents a common and increasing habit in Sweden. It has been suggested as a potential, modifiable risk factor of T2D. However, more detailed information on effects of different types of alcoholic beverages as well as the effects on early phases of T2D development seems warranted. The aim of the present study was to investigate the influence of alcohol consumption and specific alcoholic beverages on the risk of developing prediabetes and T2D in Swedish middle-aged men and women.

Materials and methods: The data of 2004 men and 2961 women, aged 35-56 yrs at baseline, from the Stockholm Diabetes Prevention Program was evaluated in this cohort study. All subjects had normal glucose tolerance at baseline. Logistic regression analysis was performed to estimate the risk (expressed as odds ratio (OR) and 95% CI) to develop prediabetes and T2D at follow up, 8-10 yrs later, in relation to self reported, baseline alcohol intake (converted to g/day) derived from wine, beer, dessert wine and hard liquor. Adjustment was preformed for several life style factors.
Results: When adjusted for confounders, no significant association was found with total alcohol consumption and prediabetes or T2D in both men and women. However, men showed a higher risk for T2D when having a high liquor consumption (OR: 2.01 (95% CI: 1.10-3.65)) and a higher risk for prediabetes when drinking high amounts of beer (OR: 1.72 (1.06-2.81)) compared to occasional drinkers. Women showed a reduced risk for T2D in the medium liquor group (OR: 0.39 (0.17-0.91)) and a 2-fold higher risk for prediabetes in the high liquor group (OR: 2.37 (1.44-3.89)) but a reduced risk for prediabetes in the high wine intake group (OR: 0.65 (0.43-0.99)) compared to occasional drinkers.
Conclusion: This is the first study to investigate the possible risk of different alcoholic beverages on the development of prediabetes in women. Although no significant associations were found to total alcohol consumption in men and women, high consumption of hard liquor and beer increased the risk of T2D development. Since alcohol in some instances reduced the risk, it is possible that other factors than ethanol might play a role.

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The inverse association between alcohol intake and complement C3 can be attributed to wine consumption and is explained by inflammation, HDL and insulin resistance, the CODAM study

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Background and aims: Moderate alcohol consumption has beneficial effects on CVD risk. Complement C3 is an early marker of insulin resistance and a risk factor for CVD and a negative association between alcohol consumption and circulating C3 has been reported. However, whether all sources of alcohol (e.g. wine, beer or other) are equally associated with C3 is not known. Also, the mechanisms that can explain any such inverse associations are not known. Beneficial effects of (moderate) alcohol consumption on inflammation, HDL and insulin resistance are likely candidates in this regard. The aim of this study was therefore to determine whether an inverse association between alcohol consumption and C3 is present and to investigate the extent to which this is (1) due to a specific source of alcohol and (2) explained by inflammation, HDL and/or insulin resistance.
Materials and methods: We investigated the Cohort on Diabetes and Atherosclerosis Maastricht (n=574, 61.3% men, 59.1 ± 7.0 yrs of age). Alcohol intake from different sources (beer, wine and others) was estimated by means of a validated food frequency questionnaire (FFQ). Subjects with unreliable FFQ (n=56) or who had missing data on the other variables (n=22) were excluded. First, we determined the association between alcohol consumption (total amount or from different sources, main determinant) and C3 (main outcome, standardized value) using linear regression (adjusted for age, sex, total energy intake, physical activity, waist, smoking, total cholesterol, ALAT, prior CVD, glucose tolerance status, use of medication). Next, we investigated whether this association could be attributed to a specific dietary source of alcohol. Lastly we investigated whether this association was (statistically) explained by inflammation (expressed as the average Z-score of CRP, IL6, SAA, sICAM, ceruloplasmin and haptoglobin), HDL-cholesterol and/or insulin resistance (as HOMA2ir).
Results: After adjustment for the above-mentioned covariates, alcohol consumption (in 10 g/d) was inversely associated with circulating C3; β [95% CI]; -0.057 [-0.100; -0.014] (p=0.010). When each source of alcohol was adjusted for each other and the covariates, only wine was independently associated with C3 (βs [95% CI] were -0.087 [-0.140; -0.033], p=0.002; -0.042 [-0.107; 0.023], p=0.222; -0.004 [-0.080; 0.072], p=0.908 for wine, beer and others, respectively). In the final analyses, we therefore focused on alcohol consumption from wine (in 10 g/d). The strength of the association between alcohol from wine and C3 was attenuated after further adjustments for inflammation (by 24.1%), HDL (by 33.3%), or HOMA2IR (by 36.8%), and was attenuated by 73.6% when these three mediators were added together.
Conclusion: The negative association between alcohol intake and circulating C3 can be mainly attributed to wine consumption and is largely explained by the favorable impact of alcohol consumption on inflammation, HDL and insulin resistance. This suggests that this association is more likely related to non-alcohol components of wine, than to the alcohol component itself.
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Steatosis of donor's liver and its relationship to post-transplant diabetes mellitus in liver transplantation

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Background and aims: Previous study indicated that HCV infection, immunosuppressant including steroid and calcineurin inhibitor, age over 45yrs, family history of DM, overweight, impaired glucose regulation before transplant, MCV infection, acute rejection(AR), cirrhosis especially decompensation cirrhosis et al were risk factors to post-transplant diabetes mellitus(PTDM). The relation between steatosis of donor's liver and PTDM was rarely reported. The aim of our study was to discuss steatosis of donor's liver and its relationship to post-transplant diabetes mellitus in liver transplantation.
Materials and methods: We retrospectively analyzed 438 patients who performed orthotopic liver transplantation (OLT) in our center between April, 2001 and December, 2008. Patients with history of using steroids, or data incomplete or died within 3 months after OLT were excluded. The grade of liver steatosis was taken pathological grading of non-alcoholic fatty liver disease (NAFLD), and fatty content less than 5% was considered without steatosis, otherwise was steatosis. Liver function was graded to A, B and C level according to Child-Pugh grade system. Patients were divided into PTDM and non-PTDM group according to fasting plasma glucose(FPG) after operation. Univariate analysis was used to analyze the possible risk factors, such as age, gender, family history of DM, HBV or HCV infection, FPG, BMI, liver function pre-operation, cirrhosis, steatosis of donor's liver, AR, immunosuppressive drugs, metabolite related risk factors, interleukin 2 receptor antagonist(IL-2RA). Multivariate logistic regression was used to analyze factors including age, FPG, HBV infection, cirrhosis, liver function pre-operation, steatosis of donor's liver, basic diseases before operation, IL-2RA, immunosuppressive drugs and AR. Student's t test (One-Way ANOVA) was used to compare quantitative variables and Chi-square test was used to compare qualitative variables. P value less then 0.05 was considered statistically significant. Statistic analysis was done by SAS 8.2 and SPSS 16.0.
Results: Among 438 patients, there were 298 non-PTDM and 140 PTDM patients. Among 298 non-PTDM patients, there were 103 steatosis of donor's liver, taking 34.6%, and 62 steatosis of donor's liver among 140 PTDM patients, taking 44.3%. Univariate analysis indicated that liver function and fasting plasma glucose pre-operation, the use of IL-2RA and calcineurin inhibitor were significantly related with PTDM (All P< 0.05), but steatosis of donor's liver was at the critical level (P=0.050). While multivariate logistic regression indicated that FPG pre-operation and steatosis of donor's liver had positive relationship with PTDM, their OR value was 1.853 (P<0.01) and 1.803 (P<0.05) respectively. And the use of IL-2RA was negatively related with PTDM with OR value of 0.427 (P<0.01).
Conclusion: We found that steatosis of donor's liver, abnormal fasting plasma glucose, liver function pre-operation and calcineurin inhibitor were risk factors of PTDM.

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B12 deficiency is more common than folate deficiency in early pregnancy: do we need to consider B12 fortification?

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Background and aims: Foetal programming due to gene-diet interactions during the periconceptional period has been linked to increased risk of Type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) in the offspring during their adult life. In addition, children who are born small but gain weight rapidly during the early years and become obese in adult life are particularly at risk of T2DM and CVD. A large prospective observational

study showed that offspring born to mothers with high folate and low B12 levels during mid-pregnancy, had the highest adiposity and greatest insulin resistance. In presence of such imbalance, it is plausible biochemically, excess energy is converted to adipogenesis as opposed to myogenesis. Though such imbalance is likely to be common in a mainly vegetarian population such as in India, recent evidence suggests that such phenomenon may not be uncommon in the UK adult population too. In animal studies, epigenetic alterations of DNA methylation by B12 and folate during the periconceptional period result in increased adiposity and metabolic risk in the offspring. In addition, methyl donor deficiency leads to dysfunction of the ghrelin system with dramatic effects on intrauterine growth. Because of fortification of various foods and the recommendation for periconceptional folic acid supplementation, folate deficiency has become rare. B12 deficiency has thus become a potentially major modifiable risk factor for metabolic disease as well as neural tube defects (NTDs). Indeed, in the presence of adequate folate, NTDs due to B12 deficiency have tripled. Thus, reducing the incidence of B12 deficiency by fortification / supplementation has the potential to reduce the risk of metabolic disease in adult life and the overall burden of metabolic disease world-wide.
To investigate the incidence of B12 deficiency in a Caucasian population during early pregnancy.
Materials and methods: 200 Maternal serum samples (mean age 27y) at 16-18 weeks gestation were analysed for B12 and folate levels using an electrochemiluminescence immunoassay. 200 samples from non-pregnant women of child-bearing age (mean 28y) were analysed as controls.
Results: B12 deficiency (<191ng/L) was common during pregnancy compared to age-matched control non-pregnant women (20% vs 4%, p=<0.0001). Folate deficiency was 6% and 13% in the pregnant and non-pregnant women respectively (<4.6μg/L). Significantly more pregnant women had folate levels above the reference range (>18.7μg/L), compared to the non-pregnant group (8% vs 3%, p=0.0283). This is likely due to increased folic acid intake during pregnancy. Median B12 levels are significantly lower (median: 262ng/L vs 363ng/L, p=<0.0001) at 16-18 weeks of pregnancy. This may be due to increased utilization during pregnancy, especially in the presence of higher folate levels.
Conclusion: These data indicate that B12 deficiency is common in early pregnancy even in a non-vegetarian UK population. If untreated, such deficiency may get worse in later pregnancy, potentially increasing the risk of metabolic disorders such as T2D and CVD. Given recent evidence linking B12 deficiency to NTDs, our findings suggest in addition to folic acid, B12 fortification should be considered. Intervention studies of B12 supplementation in early pregnancy and their effects on offspring are urgently needed. In addition, further studies designed to identify the potential mechanisms are warranted.

PS 13 Screening and prediction of type 2 diabetes mellitus

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Comparison of American Diabetes Association and World Health Organisation indications for performing oral glucose tolerance test
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Background and aims: There is a discrepancy between American Diabetes Association (ADA) and World Health Organisation (WHO) cut-off values of fasting plasma glucose (FPG) for diagnosing Impaired Fasting Glycaemia (IFG). ADA recommends an FPG cut-point for IFG of 100 mg/dl (5.6 mmol/l). WHO and the European Diabetes Epidemiology Group recommend the cut-point of 110 mg/dl (6.1 mmol/l). The aim of this study is to determine which FPG cut-point should be used when deciding whether to perform an Oral Glucose Tolerance Test (OGTT). It also aims to assess the impact of age, gender and ethnic origin on the relationship between FPG and 2-h plasma glucose (2-h PG).
Materials and methods: Our hospital serves an inner city area in London with a multiethnic population of over 250,000 people. We conducted a retrospective analysis of OGTTs performed in our institution over a 24-month period from 1st May 2006 to 30th April 2008.
Results: Data was collected on 1598 patients (mean age 58.7 years ± 13.5 years, 54.2% males and 45.8% females). Amongst the subjects there were 44.7% White, 9.9% Black, 6.2% Asian and 39.2% cases of 'other ethnic origin' or 'not stated'. Among participants with FPG of 100-109 mg/dl (normal according to WHO, but IFG according to ADA) 34.1% had impaired glucose tolerance (IGT) and 11.8% had diabetes based on 2-h PG (overall 45.9% impaired glucose regulation). In those with FPG of 110-125 mg/dl (IFG according to both ADA and WHO), 39.0% had IGT and 29.0% diabetes based on 2-h PG (overall 68.0% impaired glucose regulation). In those with FPG of <100 mg/dl (normal according to ADA and WHO), 19.3% had IGT and 4.6% diabetes based on 2-h PG (23.9% impaired glucose regulation). A statistically significant association was found between FPG and 2-h PG (p<0.001) and a positive linear relationship between FPG and 2-h PG was observed (correlation coefficient 0.436). For participants with FPG of 100-109 mg/dl, a statistically significant relationship between different age groups and 2-h PG was demonstrated (p=0.012). The proportion of participants with FPG of 100-109 mg/dl who had IGT showed a steady increase: 26.7% among <50 years of age, 29.6% among 50-60 years , 38.7% among 60-70 years , 42.4% among >70 years of age. A similar trend was noted in the proportion of individuals who were diagnosed with diabetes based on 2-h PG. For subjects with FPG of 100-109 mg/dl, no statistically significant association was noted between gender or ethnic origin and 2-h PG.
Conclusion: In this study, by applying a WHO cut-point for FPG of 110 mg/dl (6.1 mmol/l) we would have missed the diagnosis of impaired glucose regulation in a large proportion of subjects. A significant proportion of our population with FPG of 100-109 mg/dl had IGT (34.1%) or diabetes (11.8%) based on 2-h PG. This may reflect the characteristics of our local population and further studies should guide whether OGTT is indicated in individuals with FPG of 100-109 mg/dl.

Distribution of subjects by fasting and 2-h plasma glucose

FPG	<140 mg/dl(normal)	140-199 mg/dl (IGT)	≥ 200 mg/dl (diabetes)
<100 mg/dl	347 (76.1%)	88 (19.3%)	21 (4.6%)
100-109 mg/dl	219 (54.1%)	138 (34.1%)	48 (11.8%)
110-125 mg/dl	148 (32.0%)	180 (39.0%)	134 (29.0%)
≥ 126 mg/dl	24 (8.7%)	56 (20.4%)	195 (70.9%)

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Elevated one-hour post-load plasma glucose levels identifies subjects with normal glucose tolerance and impaired beta cell function, insulin-resistance and worse cardiovascular risk profile, the GENFIEV study

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Background and aims: Recent evidence suggests that in subjects with normal glucose tolerance (NGT) one-hour post-load plasma glucose (1h-OGTT glucose) >155 mg/dl may predict type 2 diabetes (T2DM) and is associated with subclinical atherosclerosis. This study evaluates beta-cell function, insulin-sensitivity and cardiovascular risk profile of NGT subjects with 1h-OGTT glucose >155 mg/dl.
Materials and methods: In 929 subjects, participating in to the GENFIEV (Genetic and Pathophysiology of Type 2 Diabetes Evolution) study, we performed an OGTT with measurement of C-peptide and fasting insulin. Insulin resistance was assessed by HOMA-IR, while beta-cell function was estimated by the Insulinogenic Index and minimal model analysis of plasma glucose and C-peptide response to a 2-hr 75-g OGTT.
Results: Based on the OGTT results, 51% had NGT, 4% IFG, 24% IGT, 7% both IFG and IGT, and 14% were diagnosed with new T2DM. Thirty-nine percent among NGT, 76% of IFG, 90% of IGT, 99% of IFG+IGT and 98% of newly diagnosed T2DM had 1h-OGTT glucose >155 mg/dl. This cutoff point has an high specificity (89%), a good sensibility (69%) and an high positive predictive power (92%) in identifying subjects with IGR or newly diagnosed T2DM. Among subjects with NGT (n. 474, 37% men and 63% women; age: 46±12 years, BMI: 28.4±5.3 kg/m2), those with 1h-OGTT glucose >155 mg/dl, were more insulin-resistant (HOMA-IR 2.7±1.9 vs 2.1±1.2 mmol/L x μU/ml; p<0.01) and had impaired first phase insulin secretion (Insulinogenic Index: 0.052±0.030 vs 0.092±0.17; p<0.01; C-CD: 1381±865 vs 1721±1384 Pmol/m2BSA/mM/min; p<0.005) and beta-cell performance (Disposition Index: 0.055±0.097 vs 0.026±0.025; p<0.001) compared to those with 1h-OGTT glucose ≤155 mg/dl. Moreover, HbA1c (5.6±0.4 vs 5.3±0.4%; p<0.0001), blood pressure (systolic: 128±13 vs 122±14 mmHg; p<0.0001 and diastolic: 81±10 vs 77±11 mmHg; p<0.0001), LDL-cholesterol (136±41 vs 127±37 mg/dl; p<0.05) and triglycerides (136±96 vs 117±75 mg/dl; p155 mg/dl, while HDL-cholesterol was lower (52±14 vs 56±16 mg/dl; p155 mg/dl had a similar cardiovascular risk profile, a comparable insulin-sensitivity impairment and a slightly better beta-cell function.
Conclusion: 1h-OGTT glucose >155 mg/dl shows a good performance in discriminating subjects with IGR/newly diagnosed T2DM and may identify, among NGT individuals, those with lower insulin-sensitivity, impaired beta-cell function and worse cardiovascular risk profile, i.e. those subjects at higher risk of developing T2DM and cardiovascular disease.
Supported by: FoRiSID with an unconditioned grant from Eli Lilly, Italy

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New and old criteria for the diagnosis of diabetes mellitus in patients with coronary artery disease

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Background and aims: Recently, an International Expert Committee concluded that haemoglobin A1c (hba1c) may be a better means of diagnosing diabetes than glucose levels. A diagnosis of diabetes was recommended with hba1c ≥6.5%. Data on the concordance of new and old criteria for the diagnosis of diabetes are very scarce; no data at all are available for patients with coronary artery disease (CAD). We therefore aimed at investigating the concordance of new and old diabetes criteria in a large cohort of patients with angiographically proven CAD.
Materials and methods: We consecutively enrolled 1124 Caucasian patients with angiographically proven CAD who did not have previously known diabetes. An oral glucose tolerance test (oGTT) was performed in all patients.
Results: From the patients with diabetes according to the new diagnostic criterion hba1c ≥6.5% (n=110), 58 (53%) fulfilled the WHO glucose criteria for diabetes, 13 (12%) had impaired glucose tolerance (IGT), 26 (24%) impaired fasting glucose (IFG), and 13 (12%) normal fasting glucose (NFG). Conversely, the hba1c ≥6.5% criterion was fulfilled in 58 patients (63%) with diabetes according to WHO criteria, in 13 patients (11%) with IGT, in 26 patients (8%) with IFG, and in 13 patients (2%) with NFG. Compared to the standard of WHO criteria, the proposed hba1c ≥6.5% for the diagnosis of diabetes had a sensitivity of 63% and a positive predictive value of 53% for detecting previously undiagnosed diabetes, whereas specificity and negative predictive value were 95% and 97%, respectively.
Conclusion: The recently recommended hba1c criterion for the diagnosis of diabetes among CAD patients is highly specific but not sensitive. This might strongly limit its use as a screening tool for identifying individuals with diabetes.

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Prevalence of diabetes mellitus after pregnancy with gestational diabetes mellitus using different cut-off criteria for abnormal glucose tolerance

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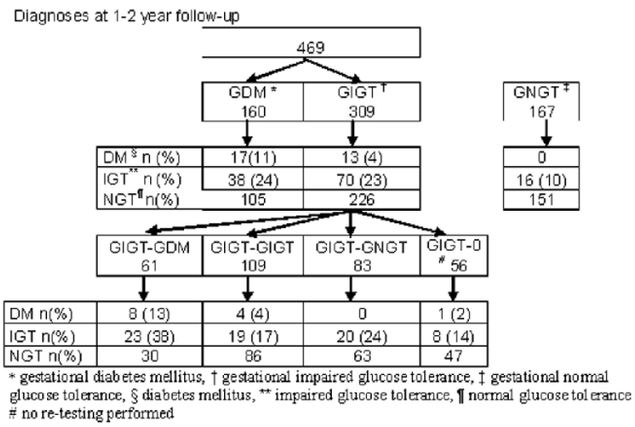
Background and aims: Gestational diabetes mellitus (GDM) is diagnosed using an oral glucose tolerance test (OGTT) but there is no consensus on diagnostic criteria. GDM is a reliably predictor for subsequent diabetes mellitus but more knowledge on the relationship between the OGTT result and future diabetes is needed. The aim was to determine the incidence of diabetes after GDM as defined by WHO 1999 using different cut-off levels for abnormal glucose tolerance during pregnancy.
Materials and methods: Women undergoing a 75 g oral OGTT during pregnancy and delivering during 2003-2005 were invited to a 5-year follow-up program postpartum. At first check-up, 1-2 years after delivery, 160 women with GDM, 309 with gestational impaired glucose tolerance (GIGT) and 167 controls with gestational normal glucose tolerance underwent an OGTT. Cut-off levels defining GDM and GIGT were 2-h capillary blood glucose levels of 9.0 and 7.8 mmol/l or plasma glucose 10.0 and 8.6 mmol/l.
Results: In addition to 15 women with GDM and 2 with GIGT diagnosed with diabetes before follow-up, 11% of women with GDM and 4% of women with GIGT were diagnosed as having diabetes, implying diabetes in 6% in the combined group. The corresponding figures for impaired glucose tolerance (IGT) were 23% and 24%. No one in the control group had diabetes whereas 10 % had IGT. Due to the low reproducibility of the OGTT women with GIGT were retested within one week, following which 24% were reclassified as having GDM, 43% with GIGT and 33% with GNGT with corresponding diabetes prevalence at follow-up of 13%, 4% and 0%.
Conclusion: Lowering the cut-off level for GDM to that proposed by WHO 1999 would substantially increase the number of women identified during pregnancy and thereby committed to special care during pregnancy and to follow-up postpartum. Lowering the threshold would dilute the diabetes prevalence and does not seem optimal to identify those at the highest risk of diabetes development. Retesting the GIGT group might offer a possible way to identify those in most need of medical attendance.



* gestational diabetes mellitus, † gestational impaired glucose tolerance, ‡ gestational normal glucose tolerance, § diabetes mellitus, ** impaired glucose tolerance, ¶ normal glucose tolerance # no re-testing performed

Supported by: foundations of the County of Skåne, Sweden

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Usual delay in sample processing can underestimate detection of prediabetes and diabetes in Korea

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Background and aims: Diabetes has emerged as a public health issue worldwide, particularly in East Asia including Korea and consensus statements recommended early detection and preventive treatment in high-risk individuals. For detection of prediabetes and diabetes(hyperglycemic state), fasting plasma glucose (FPG) alone is commonly used as a screening test but serum should not be used, because the glucose concentration decreases 5~7% per hour as a result of erythrocyte glycolysis or slow serum/plasma separation. However, since serum glucose is commonly used as screening test in Korea. The purpose of this study was to assess whether ordinary delay in sample processing influences screening results of hyperglycemic state.
Materials and methods: Between 2007 and 2009, study subjects were recruited in 2028 Korean who visited the division of endocrinology for the evaluation of the IFG previously diagnosed with the serum glucose in the health examination. We make a comparison of difference between fasting serum glucose and fasting plasma glucose, measured at the same times, and evaluated the classification of glucose tolerance determined by a 75g oral glucose tolerance test (OGTT). We also make a comparison of laboratory findings between normoglycemia and diabetes, diagnosed with plasma glucose in study populations with normoglycemia in routine chemistry. We conducted a cross-sectional study, which was approved by the Institutional Review Board of the University Hospital.
Results: We firstly obtained blood samples from another 30 adults and serum glucose concentrations were measured immediately and after 0.5, 1, 1.5, 2, 2.5and 3 hr. All the specimens were kept at room temperature during this preliminary test. The concentration of glucose declined with time (118.9mg/dL, 114.4mg/dL, 111.9mg/dL, 109.7mg/dL, 107.1mg/dL, 105.8mg/dL and 102.6mg/dL at each times) and the main decrease occurred during the first 30 min. Among 1428 persons, we recruited 1254 subjects who were diagnosed with normal fasting glucose or IFG using FPG. Mean glucose concentrations were 117.4 ± 11.3 mg/dL in plasma and 106.8 ± 8.1mg/dL using routine chemistry (mean difference: 10.6 ± 2.5 mg/dL). Of 1061 subjects diagnosed with IFG using serum glucose, 235 (22.1%) were newly diagnosed with diabetes in fasting plasma glucose and 306 (28.8%) in 75g OGTT. Age, c-peptide and insulin level at 30minutes for 75g OGTT, AST and metabolic syndrome (%) showed significant differences between normoglycemia and diabetes diagnosed with plasma glucose in 169 subjects with normoglycemia in routine chemistry.
Conclusion: This study evaluated that the ordinary delay in sample processing influences diagnosis of hyperglycemic state and our results showed that this delay can underestimate detection of hyperglycemic state, in Korea. For the screening of prediabetes and diabetes, glucose level should be measured in plasma or within 30 minutes from venous sampling.

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Increasing the flexibility of a screening model for incident diabetes only increases the predictive power marginally

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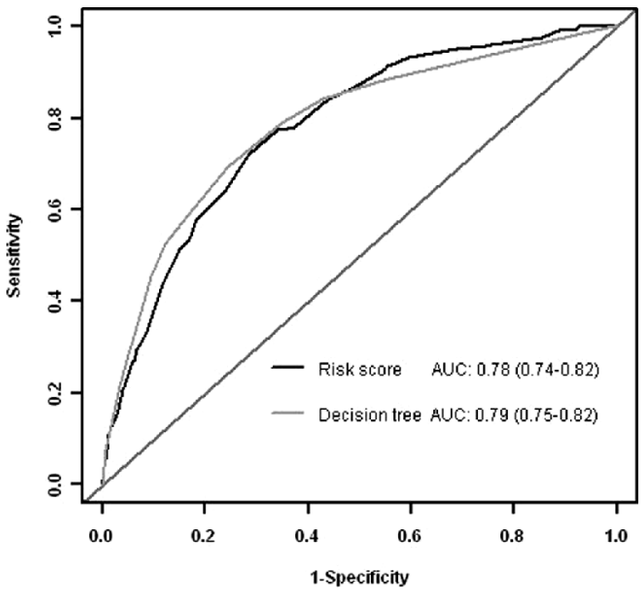
Background and aims: Risk scores for predicting diabetes tend to be simple and highly dependent on age. As such, they have limited use as a first step in a screening programme. The aim of this study was to derive a screening model based on established risk factor information obtainable through a mailed questionnaire but with a more flexible structure which accommodates the fact that some risk factors for diabetes may have different impacts in different sub-groups of people.
Materials and methods: The analysis is based on a Danish population-based primary prevention study, the Inter99 study. A total of 4,363 participants, free of diabetes at baseline and with 5-year follow-up data on diabetes status were analysed. All participants had complete information on age, sex, BMI, waist circumference, the use of antihypertensive- or lipid lowering treatment, family disposition to diabetes, smoking and physical activity. Glucose tolerance status at baseline was based on OGTTs. Incident diabetes was based on self-reported diabetes or OGTTs at follow-up examination. Two alternative screening models for incident diabetes were derived. A standard risk score assessed by Poisson regression analysis (risk score) and a more flexible model identified through tree-structured regression analysis (decision tree). The performance of the models was compared by ROC analysis.
Results: The standard risk score included information on age, sex, BMI, the use of antihypertensive- and lipid lowering treatment, parents with diabetes, smoking and leisure-time physical activity. The AUC of the standard risk score was 0.78 (95%-CI: 0.74-0.82). The decision tree included the same information as the standard model with the additional inclusion of waist circumference and had an AUC of 0.79 (95%-CI: 0.75-0.82).
Conclusion: A screening model allowing for a more flexible structure only increased the performance marginally.



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Value of the FINDRISC questionnaire to identify prediabetes in a middle sized town in Sweden

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Background: Diabetes is a common disease in the western world today, particularly in Sweden and in Finland where the prevalence of the population between 45-75 years is about 5% and 5-10%, respectively. Type 2 diabetes (T2D) is preceded by a pre-diabetic state with either impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). In this state, which may last for many years, several studies have shown that diabetes can be prevented. A combination of improved diet and increased physical activity is recognized as an efficient means to prevent development of overt T2D in subjects with IGT, but is not proved to be efficient in subjects with prediabetes/IFG. Identification of subjects with IGT is therefore of utmost importance in the strategy to prevent T2D. A questionnaire, FINDRISC, the Finnish Diabetes Risk Score, is developed and validated in Finland to identify subjects with increased risk of abnormal glucose tolerance (AGT =IFG/IGT/T2D). FINDRISC has never been evaluated in a Swedish population and is not validated to identify individuals with IGT.
Aim: To evaluate the FINDRISC questionnaire as a screening tool for diabetes and prediabetes (AGT) in an ordinary Swedish middle-aged and elderly population. In particular we wanted to identify individuals with IGT.
Materials and methods: The FINDRISC questionnaire was sent to a complete population of 5157 people, 35 to 75 years old, in a defined area in Skövde, a middle sized town of 50,000 inhabitants in the Western part of Sweden. All recipients were asked to return the completed questionnaire to the Primary

Health Care Centre at Hentorp. Maximum score was 26 and all with a self-calculated score ≥15 were encouraged to report to the clinic to have their fasting blood glucose tested. Those without diabetes were then tested with oral glucose tolerance test (OGTT) twice and participants were categorized as normal glucose tolerance (NGT), IFG, IGT, or T2D, respectively. Those with a mean 2h glucose showing IGT were invited to a diabetes prevention program.

Results: Research is ongoing and till now 2,618 questionnaires have been returned (50,7%). Mean age was 56±11 years in women and 58±12 years in men and mean score was 8.5±5.1 in men and 8.4±4.5 in women. Of these, 260 individuals (9.9%) had a risk score ≥15 and altogether 153 have, so far, had a fasting blood glucose test taken at the health care unit. We detected 16 subjects with new T2D (10.4%), 15 with IGT (9.8%) and 47 with IFG (30.7%). A FINDRISK score ≥15 was associated with a positive predictive value (PPV) of 51% for AGT, and a PPV of 9.8% for IGT.

Conclusion: In this first Swedish study the FINDRISC questionnaire was found to have a relatively high PPV of 51% when screening for AGT, but for IGT a PPV of only 9.8% was found. IFG was by far the most common form of abnormal glucose metabolism and preventive measures for these individuals should be better defined.

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A Chinese diabetes risk score for opportunistic screening of undiagnosed diabetes and abnormal glucose tolerance

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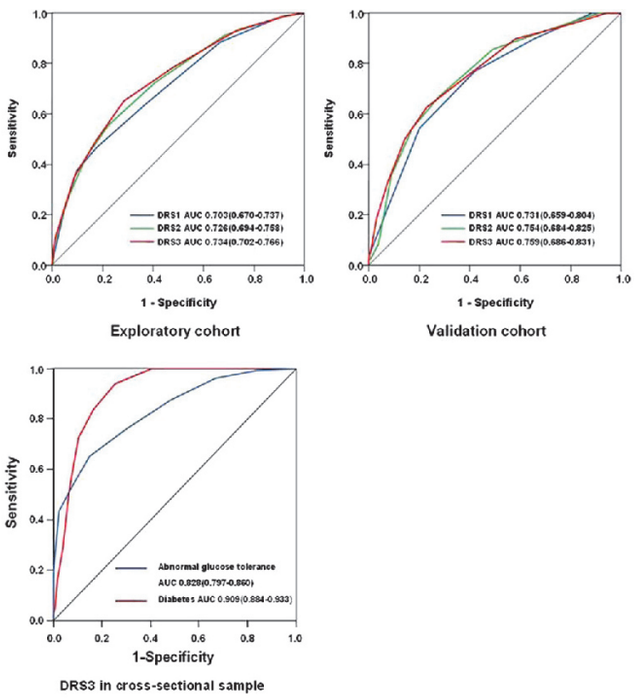
Background and aims: To develop a diabetes risk score (DRS) to predict incident diabetes and evaluate its efficiency on screening for individuals at high risk for undiagnosed diabetes and abnormal glucose tolerance in Chinese population.

Materials and methods: Three DRSs were developed based on a 10-year follow-up cohort of 1,457 individuals aged 48-87 years without diabetes at baseline and validated on another cohort of 394 non-diabetes individuals age 43-88 years, followed up after 10 years. DRS1 contained simple clinical information, while DRS2 added fasting plasma glucose based on DRS1, and DRS3 added serum lipids based on DRS2. The DRS with the largest area under the ROC curve (AUC) was chose as the final DRS and was evaluated on screening of glucose abnormality in a cross-sectional sample of 699 individuals without known diabetes.

Results: DRS3 was considered as the final DRS because it had the best prediction property. AUC was 0.734 (95% CI 0.702-0.766) predicting diabetes within 10 years, and also had adequate performance in validation cohort (AUC=0.759 (0.686-0.831). The DRS3 had sensitivity of 64.5% and 72.9%, specificity of 71.6% and 63.9% with an optimal cutoff of 4 of 12. In the cross-sectional sample, AUCs were respectively 0.828 (0.797-0.860) and 0.909 (0.884-0.933) detecting abnormal glucose tolerance and diabetes. A two-step strategy, identifying individual at increased risk for diabetes using DRS3 as a first step, followed by OGTT performance led to the identification of 76.2% of case of abnormal glucose tolerance and 100% of cases of unknown diabetes, whereas only requiring an OGTT in 47.2% study group.

Conclusion: The diabetes risk score, including clinical information and biochemical indexes has good predictive ability for incident diabetes and is practical to screen subjects with abnormal glucose tolerance and diabetes in the general Chinese population.



ROC curves showing three DRSs predicting incident diabetes in both cohorts and DRS3 screening abnormal glucose tolerance and diabetes in cross-sectional sample

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Low self rated health is associated with impaired glucose tolerance in men

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Background and aims: Self-rated health (SRH) is an independent predictor of future mortality. Recently, the weight gain in women seems to be influenced by previous poor SRH. Poor SRH in diabetics has been related with increased mortality according to McEwen et al. We attempt to see how poor SRH is related to IGT in subjects who are not aware of having it, in a Swedish population.

Materials and methods: This investigation is based on data from a population-based study in Vara(1811) and Skövde(1005) including subjects aged 30-74 years (1416 women and 1400 men) in two small communities in a rural area of south-western Sweden, as part of the Skaraborg Project. SRH was investigated by the question: "How do you rate your current health status in general?" with reply alternatives very good, good, reasonable, bad and very bad (called SRH-5 in the following text). Only a few participants rated their own health bad and very bad (fig 1). The variable SRH-5 was dichotomised in 2 values: High-SRH if the answer was "very good" and "good" and Low-SRH if the answer was "reasonable", "bad" and "very bad". A standard 75g oral glucose tolerance test (OGTT) was performed in each participant without known diabetes. Blood samples at fasting, 10, 30, 60 and 120 minutes after glucose administration were collected and analysed for p-glucose and s-insulin. IFG, IGT and diabetes was defined concordant with WHO criteria. HOMA-IR was calculated by formula HOMA-IR= FPG X FP insulin/22.5. Subjects with diabetes (108) were excluded from our analysis. Average weekly alcohol consumption, physical activities (PA) and sleeping disorders were estimated in a structured questionnaire. Logistic regression analysis has been used on estimating dichotomised variables and Univariate Analysis of Variance was used to estimate mean differences for continuous variables in subjects with low respective high SRH. Results were calculated separately in men and women.

Results: According to criteria described on methods 72.6 % rated high his own health and 26.3 % low (in 30/2816=1.1% missing). An evaluation av mean differences between subjects with low respective high SRH showed that low SRH is associated with higher BMI, fasting glucose, fasting insulin and HOMA-IR. Low SRH was also associated with higher incidence of IGT(M:

5.2% resp 13.4% Or 2,83 CI 1.8-4.4 p<0,001; F 8.5% resp 12.4% Or 1.52 CI 1,0-2.2 p=0.033). Adjusting for possible confounders age, BMI, PA, alcohol consumption and sleeping disorders we found a remaining strong association between SRH and IGT in males but not in females (Tab.1).
Conclusion: These results confirm IGT to influence independently the perception of health even when it is an unknown state. Nevertheless there are some gender differences suggesting ulterior confounders in men, in addition to those we reported. On the other hand SRH is confirmed an independent risk factor to disease which confirm the importance of using it on daily practice.

Tab 1. Logistic regression analysis adjusted for possible confounders in males.

Males	High SRH	Low SRH	OR/Δ mean	CI	P-value	P-value
IGT	5.2%	13.4%	2,83	**1.8-4.4**	**0.000**	**0.000**
Adj. for age			2.83	**1.8-4.5**	**0.000**	**0.000**
Adj. for age and BMI			2.51	**1.6-4.1**	**0.000**	**0.000**
Adj. for age and PA			2.36	**1.4-3.9**	**0.001**	**0.001**
Adj. for age and sleep			2.76	**1.7-4.6**	**0.000**	**0.000**
Adj. for age and alcoh			2.88	**1.8-4.7**	**0.000**	**0.000**
Adj. for all conf together			1.95	**1.1-3.4**	0.017	0.017

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Predictors of normalisation of prediabetes and of persistence of normal glucose tolerance: KORA S4/F4 cohort study
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Background and aims: Reversion from prediabetes to normal glucose tolerance (NGT) without specific interventions has been rarely studied. We investigated in a cohort study which factors beyond blood glucose (lifestyle, clinical parameters) are associated with normalization of glucose tolerance. In addition, we investigated which factors contribute to long-term persistence of NGT.
Materials and methods: Oral glucose tolerance tests were conducted at baseline and at follow-up in a population-based study in Southern Germany (KORA S4/F4; 1,223 non-diabetic subjects aged 55-74 years at baseline (1999-2001); 887 subjects (73%), 436 of whom had prediabetes at baseline, participated in the 7-year follow-up). Prediabetes comprised impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) (ADA diagnostic criteria). Subjects who were prediabetic at baseline and normoglycaemic at follow-up were only classified as "return to NGT" when their fasting plasma glucose decreased by > 5 mg/dl or when their 2 h glucose decreased by > 10 mg/dl (clinically relevant differences).
Results: 66 of 436 (15%) subjects who had been prediabetic at baseline returned to NGT after 7 years. In a logistic regression model with age, sex, BMI, change of BMI, glucose values, diabetes family history, and lifestyle factors as independent variables, fasting and 2h glucose were strong predictors of normalization of prediabetes (p<0.001 both). Women were more likely to return to NGT (OR=2.6, 95%-confidence interval (CI)=1.3-5.0). A decrease in BMI significantly improved the chance of returning to NGT (OR, 95%CI: 7.7, 3.0-20.1 for BMI change <= -1kg/m^2, and 3.6, 1.6-8.2 for -1kg/m^2 < BMI change <= 1kg/m^2, compared to BMI change > 1kg/m^2) whereas baseline BMI was not independently associated with return to NGT (p=0.57). Physical activity, smoking and alcohol intake were not significantly associated with return to NGT. After replacing BMI by waist circumference (WC), an increase in WC turned out to diminish chances of return to NGT (OR: 0.7, 0.5-0.9 per 5 cm). Moreover, subjects with hypertriglyceridaemia (triglycerides >= 2.0 mmol/l) and subjects with low HDL-cholesterol (men: <= 1.03 mmol/l, women: <= 1.29 mmol/l) were less likely to become normoglycaemic (p<0.05). Among 451 subjects with NGT (baseline) 321 (71%) remained normoglycaemic in the 7-year follow-up. In a logistic regression model with age, sex, BMI, glucose values, family history, and lifestyle factors as independent variables, higher levels of 2h glucose (p<0.001), higher BMI at baseline (OR: 0.93, 0.88-0.99 per BMI unit), high alcohol consumption (OR: 0.5, 0.3-0.97 compared to moderate consumption), and parental diabetes (OR: 0.5, 0.3-0.9) significantly decreased the likelihood of NGT persistence. For BMI change, a borderline significance was found (OR, 95%CI: 1.9, 0.99-3.8 for BMI change <= -1kg/m^2 compared to BMI change > 1kg/m^2).
Conclusion: Reversion to NGT without specific interventions is not a rare event in older prediabetic subjects. Several factors modifiable by lifestyle (in particular weight change) have an influence on normalization of prediabetes as well as on persistence of NGT.
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Assessment of glycated haemoglobin A_{1c} as a potential diagnostic tool in prediabetes and diabetes

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Background and aims: During recent years there is an increasingly recognized need to develop strategies and criteria for diabetes screening and diagnosis that will allow effective early disease detection and will find out the possible utility of glycated hemoglobin A_{1c} (HbA_{1c}). The aim of the present study is to measure the level of HbA_{1c} in subjects with different glucose tolerance - normal glucose tolerance (NGT), impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and newly-diagnosed diabetes (NDD) and to evaluate the potential role of HbA_{1c} as a diagnostic tool for undetected diabetes and prediabetes (IFG and IGT).
Materials and methods: A total of 2134 subjects (899 males and 1235 females), of mean age 50.3±13.9 years and mean BMI 29.5±6.2 kg/m^2 were included in the study. According to their glucose tolerance they were divided into 4 groups - 1198 with NGT, 313 with IFG, 241 with IGT and 382 with newly-diagnosed screening-detected type 2 diabetes. All participants underwent a standard oral glucose tolerance test (OGTT) with 75g glucose and the categories of glucose tolerance were defined according to 2006 WHO criteria. Plasma glucose during OGTT - fasting and 2-hour level, was measured by a hexokinase enzyme method. HbA_{1c} was measured by an immunoturbidimetric method (COBAS INTEGRA 400, Roche Diagnostics GmbH, Mannheim, Germany). Statistical analysis of the data was performed by SPSS 16.0 for Windows (SPSS, Chicago, USA). Receiver operating characteristic (ROC) curve analysis was used to examine the sensitivity and specificity of HbA_{1c} for detecting diabetes and prediabetes.
Results: HbA_{1c} levels were significantly higher in all groups with altered glucose tolerance - 5.72±0.61% in IFG, 5.84±0.63% in IGT and 7.5±1.69% in NDD as compared to the group with NGT - 5.23±0.65% ($p<0.0001$ for all groups). There was significant difference in HbA_{1c} between the two prediabetic states ($p=0.02$), the level of HbA_{1c} of both groups being significantly lower as compared to NDD ($p<0.0001$). Significant positive correlation was established between the level of HbA1c and both fasting plasma glucose ($r=0.78$, $p<0.001$) and 2-hour plasma glucose ($r=0.76$, $p<0.001$). The ROC analysis demonstrated that HbA_{1c} had strong correlation with undiagnosed diabetes, with an area under the receiver operating characteristic curve (AUC-ROC) of 0.958 (95% CI: 0.946-0.970), as well as with undiagnosed prediabetes - AUC-ROC of 0.729 (95% CI: 0.702-0.755). The AUC-ROC for undiagnosed DM was similar between HbA_{1c} and fasting plasma glucose - 0.99 (95% CI: 0.983-0.997) and 2-hour plasma glucose - 0.982 (95% CI: 0.973-0.992). Analysis with ROC curves showed that the optimal cut-off level of HbA_{1c} for diagnosis of diabetes was 6.1% with a sensitivity of 86% and specificity of 92%. The optimal cut-off level of HbA_{1c} for undiagnosed prediabetes (IFG and IGT) appeared to be 5.5% with a sensitivity of 71% and specificity of 64%.
Conclusion: HbA_{1c} appears to be a sensitive and useful tool for identifying subjects with impairment in glucose tolerance (prediabetes and diabetes) and it should be considered in the development of diagnostic strategies.
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The role of haemoglobin A_{1c} testing in diagnosing diabetes in Korean adults

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Background and aims: The international expert committee has recently recommended the use of HbA1C assay to diagnose diabetes, with a threshold of ≥ 6.5%. We aimed to characterize the cut off value of HbA1C in diagnosing diabetes based on 75-g oral glucose tolerance test (OGTT) in Korean adults.
Materials and methods: We recruited 902 adults (mean age 40±13(21~80) yrs, mean BMI 22.9±3.5 kg/m^2) without a self-reported history of diabetes from 8 university hospitals in 2009. A 75-g OGTT and HbA1C sampling were performed in all examinees. Glucose concentrations were measured by colorimetry method (ADVIA2400 autoanalyzer, Siemens, USA), and HbA1C was measured by immunoturbidimetric method (Cobas integra800, Roche, Switzerland) at the central laboratory. Receiver operating characteristic curve analysis was used to examine the sensitivity and specificity of HbA1C for diagnosing diabetes.
Results: The HbA1C threshold of 6.0% proved to be the optimal limit for diagnosing diabetes, with a 88.2% sensitivity and a 79.9% specificity. The cut off values increased with age (6.0% at ages 21~40, 6.2% at 41~60, and 6.4% at > 61 years) and were similar in both men and women (6.2 vs. 6.1%). HbA1C of ≥6.5% had a 53.8% sensitivity and a 98.3% specificity in detecting diabetes. This relatively low sensitivity may limit its use as a screening method for diabetes. Using the HbA1C ≥ 6.5% criteria, the positive predictive value (PPV) of OGTT based diabetes was 74.6%, with a negative predictive value (NPV) of 95.2%. And using the HbA1C ≥ 6.0% criteria, the PPV of OGTT based diabetes was 74.6%, with a NPV of 95.2%.
Conclusion: From our study, the cut-off value of HbA1C for diagnosing diabetes based on 75-g OGTT was 6.0%, and adjustment of this value by age would be needed. Further studies will be carried out to determine whether age-specific diagnostic criteria should be needed.

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Screening for diabetes; inappropriate classification of "low risk"?

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Background and aims: Recently there has been debate regarding screening strategies for type 2 diabetes (T2DM). Latest guidelines from the American Diabetes Association (ADA) and from the UK Department of Health in the United Kingdom (DoH) recommend the use of glycolysated haemoglobin (HbA1c) as a method of diagnosing T2DM in patients and also identifying patients at risk of developing the condition in the future. The threshold of 6.5% has been agreed upon by both parties as the diagnostic threshold for the diagnosis of T2DM. In the identification of patients at high risk of T2DM, the ADA has proposed an HbA1c of equal to or more than 5.7% as compared to DoH of 6.0%. Patients considered at high risk of developing DM should be offered diet/lifestyle interventions and recommended for regular screening in the future. This study sought to study the patients who had an HbA1c and a concurrent glucose tolerance test (OGTT) and to study the relationship between the two results with regard to these new proposed thresholds.
Materials and methods: During the period Jan 2007 to Nov 2009 inclusive, all patients who had an OGTT and HbA1c performed concurrently within our primary care trust were included in the analysis. Data was obtained from the laboratories services serving the area. All HbA1c results were calibrated through harmonisation to the DCCT (NGSP) assay. All patients tested for gestational diabetes or were pregnant were excluded from the analysis. The results were then stratified into groups with HbA1c of less than 5.7%, between 5.7 and 5.9% and more than 6.0% and OGTT result of either a fasting glucose of ≥7 mmol/L or a 2 hour reading of ≥ 11.1 mmol/L.
Results: There were a total of 835 patients who fit the criterion for analysis. (444M, 391F, median age 64 years (interquartile range 56-73). Using the ADA cut off of an HbA1c of less than 5.7%, 4.0% of all patients screened had an OGTT consistent with diabetes. Increasing the cut off to less than 6.0% (DoH guidelines) results in a further 58 patients (6.9% of all patients screened), i.e. in total 91 patients (10.9% of all screened) who would have been stratified as low risk despite their abnormal OGTT.
Discussion: In summary using a current ADA cut off of HbA1c 5.7% in the identification of patients at risk of developing DM fails to identify approximately 1 in 25 patients who may have overt diabetes mellitus. The DoH cut off of 6.0% results in a further 1 in 15 of patients who were classified as low risk. This results highlight a clinical concern as these patients may not receive another screening appointment for a prolonged period despite results which previously would have resulted them receiving appropriate intervention.

Table 1

	HbA1c < 5.7%	HbA1c 5.7-5.9%	HbA1c ≥ 6.0%
Normal OGTT	88 (10.5%)	98 (11.7%)	119 (14.3%)
Abnormal OGTT	33 (4.0%)	58 (6.9%)	439 (52.5%)
Total	121	156	558

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Screening diabetes with HbA_{1c} and fasting plasma glucose

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Background and aims: To examine the validity of glycated Haemoglobin Alc and fasting plasma glucose (FPG) as a screening test for type 2 diabetes.
Materials and methods: A total of 1330 Chinese subjects (433 male and 897 female) at an average age of 58.23±12.75 were enrolled. All subjects underwent a 75g oral glucose tolerance test (OGTT) and AlC measurement. Receiver operating characteristic curve (ROC curve) analysis was used to examine the sensitivity and specificity of FPG and Alc for detecting diabetes.
Results: Based on 1999 WHO criteria,944 had normal glucose tolerance(NGT),22 had impaired fasting glucose(IFG),236 had impaired glucose tolerance(IGT),37 had both of IGT and IFG,91 had diabetes. The prevalence of newly diagnosed diabetes was 6.84%. Based on the ROC curve, the optimal cut-point of FPG related to diabetes diagnosed by OGTT was 5.77 mmol/L which was associated with a sensitivity and specificity of 85.7% and 91.2% respectively. The area under the curve (AUC) is 0.923 (95%CI 0.881~0.964). The positive likelihood ratio is 9.74 while the negative likelihood ratio is 0.16. The optimal cut-point of Alc related to diabetes diagnosed by OGTT was 6.1%,which was associated with a sensitivity and specificity of 83.5% and 88.88% respectively. The area under the curve (AUC) is 0.905 (95% CI0.861~0.950). The positive likelihood ratio is 7.46 while the negative likelihood ratio is 0.19. The optimal cut-point of FPG related to IGT was 4.98mmol/L which was associated with a sensitivity and specificity of 65.2% and 65.2% respectively. The area under the curve (AUC) is 0.718(95%CI 0.684~0.752). The positive likelihood ratio is 1.87 while the negative likelihood ratio is 0.53. The optimal cut-point of HbAlc related to IGT was 5.7% which was associated with a sensitivity and specificity of 65.6% and 63.1% respectively. The area under the curve (AUC) is 0.680(95%CI 0.643~0.717). The positive likelihood ratio is 1.77 while the negative likelihood ratio is 0.55.
Conclusion: Compared with A1c, FPG has a greater value in diabetes screening, they have relativity in sensitivity, specificity, positive likelihood ratio and negative likelihood ratio. The subjects with HbA1c ≥ 6.1% or FPG ≥ 5.77mmol/L should be tested by OGTT to identify if they have diabetes or not.

Comparisons of the sensitivity and specificity with different diagnosis values for diabetes

Standard	Sensitivity (%)	Specificity (%)	Positive likelihood ratio	Negative likelihood ratio
FPG≥5.60mmol/L	85.7	88.5	7.45	0.16
FPG≥7.00mmol/L	62.6	100	∞	0.37
FPG≥5.77mmol/L	85.7	91.2	9.74	0.16
bA1c≥6.1%	83.5	88.8	7.46	0.19
FPG≥5.77mmol/L and HbA1c≥6.1%	78.0	95.2	16.25	0.23
FPG≥5.77mmol/L or HbA1c≥6.1%	93.4	78.6	4.36	0.08

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Limitations of HbA_{1c} as a diagnostic test

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Background and aims: Recent ADA recommendations have included glycated hemoglobin as a new diagnostic criteria for diabetes mellitus. However, glycation of hemoglobin is a complex process influenced by hereditary, racial and environmental factors as well as the hemoglobin turnover. Together, only about 50% of the variation in glycated hemoglobin levels is expected to be explained by blood glucose profiles. In this study, we have therefore compared the diagnostic value for diabetes of glycated hemoglobin and fasting and stimulated blood glucose concentrations after an oral glucose tolerance test according to the new ADA recommendations in a risk cohort.
Materials and methods: 2036 previously non-diabetic Caucasians at risk to develop type 2 diabetes consecutively underwent a 75g oral glucose tolerance test. Glycated hemoglobin was determined with the HPLC method (Tosoh A1c 2.2), external and internal quality controls were well within the allowed ranges.
Results: The oral glucose tolerance test classified 1523 individuals as *normal* glucose tolerant (NGT), 387 as impaired glucose tolerant (IGT) or impaired fasting glycemia (IFG) and 126 as diabetic. Using the newly recommended glycated hemoglobin cut-off for diabetes of 6.5%, 53 % of the diabetic individuals were not detected. In our cohort, 2-h plasma glucose but not fasting glucose identifies 65% of all diabetic patients with glycated hemoglobin <6.5%. 39% of the diabetic patients' glycated hemoglobin was in the intermediate range of 5.7-6.5% . To diagnose these patients, one third of the total cohort with a glycated hemoglobin in the intermediate range would need to undergo a re-screening by an OGTT. Still, one in seven diabetic subjects had a glycated hemoglobin below 5.7% and would remain undiagnosed.
Conclusion: Aiming to prevent glucotoxic beta-cell destruction at an early stage of the disease, increased postprandial blood glucose values need to be diagnosed and treated. Therefore, despite the intriguing simplicity, glycated hemoglobin has obvious limitations to diagnose diabetes and prevent its complications.

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The HbA_{1c} cut points for detecting type 2 diabetes and prediabetes in a risk population - is this the right way?

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Background and aims: The study's aim was to evaluate the adequacy of a HbA1C cut point as screening tool to detect type 2 diabetes (T2DM) and prediabetic stages in a risk population for diabetes.
Materials and methods: A total of 1,028 middle aged (40-70 years) German participants of the Risk factors in IGT for Atherosclerosis and Diabetes study (RIAD) without known diabetes were included. A standardized 75g OGTT was performed after an overnight fasting period. As the basis for the classification of diabetes and its prediabetes stages it was applied the definition by WHO. Plasma glucose was measured by hexokinase method and HbA1C by HPLC. Based on the method of the receiver operating characteristic curve (ROC) the optimal cut point for T2DM, impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) with sensitivity and specificity was calculated.
Results: The prevalence of newly detected T2DM was 13.8%, of IGT 25.7% and IFG 10.6%. The cut points with the corresponding values for sensitivity and 1-specificity were prepared in the table.
Conclusion: The results of the HbA1C measurement (as screening tool for newly diagnosed T2DM and the prediabetic stages) suggest in this risk population a sensitivity of approx. 65% but a high rate of false positive tests between 21 and 48%. This indicates that HbA1C could not appropriate to detect T2DM and prediabetes in a risk population. The physicians should assess the individual risk of the patient and in case of high risk should perform an OGTT to evaluate the glycemic state.

HbA1C cut points for the hyperglycemic stages

	HbA1C cut point value (%)	AUC	Sensitivity	1-specificity
T2DM	6.0	0.80	0.66	0.21
IGT	5.6	0.62	0.67	0.48
IFG	5.6	0.65	0.63	0.44

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The HbA_{1c} assays in population diabetes screening in Eastern Poland

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Background and aims: The glycosylated haemoglobin (HbA1c) assay is the test of choice for the metabolic control of diabetes (DM). In recent time it is recommended also for its diagnosis. The aim of the study was to evaluate the diagnostic utility and limitations of HbA1c in screening for DM in adult population of Eastern Poland.
Materials and methods: In the representative sample of adult population of the Lublin region chosen from the general population by two layer drowning, the oral glucose tolerance test (OGTT) , estimation of blood lipids , evaluation of blood pressure , body mass index (BMI) at baseline (1999-2001), and at 5th yr follow-up (2004-2005) were performed. DM was diagnosed according to WHO criteria. HbA1c was determined in 2170 of 3781 survey par-

ticipants (mean age 55,9±12,3) at baseline screen and it was repeated in 386 subjects in follow-up. HbA1c assay was performed by LPLC chromatography standardized by NGSP. The diagnostic usefulness of HbA1c was evaluated using ROC (Receiver or operating characteristic) curve analysis.
Results: The mean value of HbA1c in 1294 subjects with normal OGTT was 5,4± 0.54% ,in 617 subjects with impaired glucose tolerance- 5,55 ±0.6%, in 214 subjects with newly detected DM - 5,96 ±1.13%, and in 40 - with known DM- 7,1 ±1.64% .The HbA1c didn't change significantly in a group of 253 subjects during 5 year long prospective observation (5,51±0,48 vs 5,49±0,51%). In follow-up study 240 newly detected diabetics were recognised. The baseline cut- off point of HbA1c in ROC curve for future diabetes was estimated at 5,3% (75,2% sensitivity, 50,1% specificity). The HbA1c value ≥6.5% was found in 154 subjects (14,1% of studied cases). Among them 65 subjects were diagnosed by WHO criteria as diabetic and 52 subjects had DM diagnosis by ADA criteria. As the cut-off point of 6.5% is related more to the risk of diabetic complications than single measures of glucose concentrations, the ADA criteria didn't captured 66%, and WHO criteria -58% of high risk individuals. The sensitivity/specificity of A1c cut-off point of 6,5% was in our population 16/97%.
Conclusion: Our results are consistent with data from US population study with comparable sample size and similar age. The diagnostic utility of HbA1c with recommended cut-off point of 6,5% is limited by low sensitivity of test assessed in polish population on 16%.
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Detection of diabetes in coronary artery disease: oral glucose tolerance test or glycated haemoglobin and fasting plasma glucose?
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Background and aims: The proposal of the ADA of incorporating the glycated hemoglobin (A1C) in the diagnosis of the newly detected diabetes (NDD) may interfere with the recommendation of the EASD about performing the oral glucose tolerance test (OGTT) in patients with coronary artery disease without known diabetes. We sought to determine the impact of both test, A1C and OGTT, in the diagnosis of diabetes in our series.
Methods: We analysed 338 patients with coronary artery disease without known diabetes treated with percutaneous intervention. Two weeks after discharge an analysis including fasting plasma glucose (FPG), OGTT and A1C was performed. Newly detected diabetes was diagnosed by FPG if glucose ≥126 mg/dl; by A1C if FPG< 126 mg/dl and A1C≥6.5% and by OGTT if FPG< 126 mg/dl and A1C< 6.5% and glucose post-challenge ≥200 mg/dl.
Results: Age 66.5 (56-74), males 80.1%, hypertension 49.7%, obesity 35.5%, previous myocardial infarction 37.3%. After the analysis the metabolic profile of the series was: NDD 77 patients (22.8%), prediabetes 146 (43.2%) and normoglycemic 115 (34%). Of the totality of patients diagnosed of NDD, in 19 (24.6%) the diagnosis was by FPG, 5 (6.4%) with the A1C and 53 (69%) by OGTT.
Conclusion: In our series a screening of diabetes in patients with coronary artery disease based in the FPG and A1C only diagnoses 31% of the real NDD. The OGTT is still absolutely necessary to rule out the NDD in this population.
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Proposal to rule out the unknown diabetes in patients with coronary disease
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Background and aims: The EASD recommends to perform an oral glucose tolerance test (OGTT) to all the patients with coronary disease without known diabetes. This proposal needs to be balanced with three facts: the OGTT is not usually performed in daily practice, the key in this population is the diagnosis of unknown diabetes (UDM) because the presence of prediabetes would not substantially modify its secondary prevention and lastly the inclusion of the glycated hemoglobin (A1C) in the diagnosis of diabetes. We sought to validate a new score to rule out the presence of UDM in patients with coronary disease, selecting the indication of the OGTT.
Materials and methods: In a cohort of 338 patients without known DM, we perfectly characterized the glycometabolic profile with fasting plasma glucose (FPG), OGTT, A1C and insulinemia, the coronary risk factors and the extension of the coronary disease. With a logistic regression analysis the predictors of UDM by the OGTT (defined as glucose postchallenge>200 mg/dl) were determined and a score was assigned to each patient.
Results: Seventy-seven of the 338 patients presented UDM, 146 prediabetes and 115 were normoglycemics. Thirty-one percent of the UDM could be diagnosed only with the FPG and A1C. The predictors of UDM in OGTT were: Age> 65 years (OR 2.8 (1.2-5.2), p=0.015, 3 points), non-coronary vascular disease (OR 2.6 (1.2-5.9) p=0.018, 3 points), ejection fraction<45% (OR 2.7 (1.03-7) p=0.044, 3 points), FPG> 100 mg/dl (OR 4.74 (2.4-9.5) p<0.001, 5 points) and A1C>6.1% (OR 5.8 (1.5-21.7) p=0.009, 6 points). The best cut-off point was established in >6 points (AUC 0.80, CI 95% (0.74-0.87) p<0.001). Thus, performing the OGTT to 31% of the population and together with the diagnosis by FPG and A1C it is possible to localize 83% of the real cases of UDM.The score was validated in another series of 115 patients with very close reproductability (AUC 0.84, CI 95% (0.74-0.95) p<0.001).
Conclusions: A systematic screening with FPG and A1C and performing the OGTT only depending on the risk assessed by our score (31% of the population) allows the diagnosis of 83% of the UDM.
Supported by: Spanish Society of Cardiology

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Differences in cardiovascular risk profile of diabetic subjects discordantly classified by diagnostic criteria based on glycated haemoglobin and the oral glucose tolerance test
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Background and aims: Last year, an International Expert Committee advocated the use of glycated haemoglobin A1C testing for diagnosis of diabetes. Based on the correlation between A1C levels and risk of retinopathy in several epidemiological studies, the committee determined that an A1C value of 6.5% or greater should be used as the diagnostic threshold. Guided by this report, several leading organizations, including the American Diabetes Association (ADA), have approved the use of A1C as an additional criterion for diagnosing type 2 diabetes. The present study assesses the differences in the cardiovascular risk profiles of subjects differently categorized as having or not having diabetes with diagnostic criteria based on plasma glucose and A1C proposed by the 2010 American Diabetes Association clinical practice recommendations.
Materials and methods: A standard oral glucose tolerance test (OGTT), A1C, and a set of cardiovascular risk factors and indirect measures of insulin resistance and insulin secretion were assessed in 964 individuals without previously known diabetes participating in the Telde Study, a cross-sectional epidemiological survey in Gran Canaria, Canary Islands, Spain.
Results: Taking the OGTT as the golden standard, the sensitivity and specificity of an A1C value ≥ 6.5% were 38.7% and 99.6%, respectively. Only four subjects diagnosed with diabetes by A1C did not also fulfil OGTT-based diagnosis. Those who met both diagnostic criteria presented greater measures of BMI and waist circumference, and higher values for fasting and 2-h plasma glucose, HOMA-IR, plasminogen activator inhibitor-1 and fibrinogen than subjects with diabetic OGTT but A1C < 6.5%. Abdominal obesity and 2 hours plasma glucose were the only variables independently associated with an A1C value ≥ 6.5% in a multivariate regression analysis.
Conclusion: Newly diagnosed diabetic individuals who fulfill both glucose and A1C-based diagnostic criteria for the disease seem to display a more unfavorable cardiovascular risk profile than individuals who meet the glucose-based but not A1C-based criteria.

PS 15 Anthropometric and clinical predictors of type 2 diabetes mellitus

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Prediction of incident type 2 diabetes in Norwegians using simple anthropometric measures - the HUNT study

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Background and aims: Waist circumference cut-points have been used to indicate increased risk of diabetes and cardiovascular disease. The cut-points for Caucasians have been recommended, however, based on limited data. Moreover, it is still uncertain if measures of abdominal obesity such as waist circumference and waist-to-hip ratio are superior at predicting diabetes risk over body mass index. The aims were, therefore, to establish which simple anthropometric measure best predicts incident type 2 diabetes, and to determine the optimal cut-points of each anthropometric measure for the prediction of incident type 2 diabetes in Caucasians.
Materials and methods: Participants without known diabetes at the Norwegian HUNT second survey (HUNT 2) who also participated in the third survey (HUNT 3), with height, weight, waist and hip circumferences measured at HUNT 2, and diabetes outcome at HUNT 3 were included. Receiver operating characteristic curves were used to assess discrimination of type 2 diabetes, separately, for men and women.
Results: A total of 34293 participants (55% female) with 1176 new cases of type 2 diabetes were included in the analyses. The area under the curves for waist-to-height ratio (0.783 for men; 0.821 for women) and waist circumference (0.768 for men; 0.810 for women) were larger than for body mass index (0.760 for men; 0.797 for women). The optimal cut-points for men were 27.8 kg/m^2 for body mass index (sensitivity 67%; specificity 71%), 95 cm for waist circumference (sensitivity 70%; specificity 70%), 0.90 for waist-to-hip ratio (sensitivity 76%; specificity 62%), and 0.53 for waist-to-height ratio (sensitivity 71%; specificity 71%). For women, the respective cut-points were 26.8 kg/m^2 (sensitivity 81%; specificity 66%), 85 cm (sensitivity 76%; specificity 71%), 0.81 (sensitivity 76%; specificity 66%), and 0.51 (sensitivity 81%; specificity 68%).
Conclusion: Measures of abdominal obesity were superior to body mass index in the prediction of type 2 diabetes. There was, however, no statistical difference to suggest clinical advantage of one measure over another. The optimal cut-points for body mass index in both sexes and for waist circumference in women were higher than those currently applied to Caucasians. Hence, the current anthropometric cut-points for Caucasians, or at least for Norwegians, may be inappropriate. These results should inform risk prediction algorithm in Caucasians.
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Waist circumference and body mass index as predictors of prediabetes and type 2 diabetes in middle-aged Swedish women and men

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Background and aims: Obesity is an important risk factor for type 2 diabetes (T2D). Waist circumference (WC) and body mass index (BMI), measurements of central fat distribution and general obesity, respectively, are used to predict T2D. Recently, in a 7-yrs follow-up study of older subjects (mean age 69 yrs at baseline) it was shown that WC and BMI yielded similar prediction in men, whereas WC was a superior predictor of T2D in women. The aim of the present population-based study was to evaluate WC and BMI as predictors of T2D, as well as of prediabetes (IFG, IGT), in a cohort of younger Caucasian subjects and in separate analysis for men and women.
Materials and methods: Subjects having normal glucose tolerance (NGT) at baseline, 3190 women (48±5 yrs, mean±SD) and 2215 men (47±5 yrs), participants of Stockholm Diabetes Prevention Program, were again at follow-up, 8-10 years later, investigated with an oral glucose tolerance test (OGTT). T2D, including also those who were diagnosed with T2D during the time period between baseline and follow-up, IFG and IGT was detected in 59 (1.8%), 41 (1.3%) and 120 (3.8%) women and in 107 (5.0%), 81 (3.7%) and 126 (5.7%) men, respectively. Logistic regression analysis was performed and receiver operating characteristic (ROC) curves, with corresponding area under curve (AUC), evaluated the predictive power of WC and BMI, measured at baseline.
Results: In univariate analysis, both WC and BMI were strongly associated with the development of IFG, IGT and T2D in women and men, p<0.001. In women, WC and BMI predicted IFG similarly, ROC-AUC was 0.73 (0.67-0.81, 95% CI) for WC and 0.75 (0.69-0.81) for BMI. However, for IGT and T2D WC was a stronger predictor in women; ROC-AUC was 0.70 (0.65-0.75) as compared to 0.67 (0.62-0.72), p=0.034, for IGT, and corresponding values for T2D were 0.80 (0.74-0.86) vs. 0.76 (0.69-0.83), p=0.010. In men, BMI was superior to WC in the prediction of IFG, ROC-AUC was 0.65 (0.59-0.70) vs. 0.59 (0.53-0.65), p=0.005, whereas the two measurements predicted similarly for IGT and T2D. The BMI ROC-AUCs were 0.67 (0.62-0.72) and 0.68 (0.63-0.73) and the WC ROC-AUCs were 0.65 (0.59-0.70) and 0.67 (0.61-0.72) for IGT and T2D, respectively. The optimal cut-off point for WC in the prediction of T2D, using optimal sensitivity and specificity, was 83 cm in women and 96 cm in men.
Conclusion: In middle-aged men BMI is equal or better than WC in the prediction of prediabetes and T2D. In contrast, in women WC is the equal or stronger predictor. The cut-off points for WC in the prediction of T2D are close to the values of the metabolic syndrome definition according to the International Diabetes Federation.

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Optimal waist circumference cutoff value predicting the incident type 2 diabetes as a diagnostic criterion of metabolic syndrome in Korean population aged 40 years and over: the Chungju Metabolic Disease Cohort study (CMC study)

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Background and aims: In 2009, a joint statement of the International Diabetes Federation (IDF); the National Heart, Lung, and Blood Institute (NHLBI); the American Heart Association (AHA); the World Heart Federation; the International Atherosclerosis Society; and the International Association for the Study of Obesity proposed a harmonized definition of metabolic syndrome (MetS) in which the presence of any three of five risk factors comprises a diagnosis of MetS. This new definition of MetS recommends that the IDF cutoff points of waist circumference (WC) be used for non-Europeans until more data are available. Several WC cutoffs for Korean population have been proposed, however, their results have limitation of cross-sectional studies. We aimed at determining the cutoff value of waist circumference as a diagnostic criterion of metabolic syndrome with respect to its ability to predict the incident type 2 diabetes in a Korean population.
Materials and methods: The Chungju Metabolic Disease Cohort (CMC) study that began in 2003 is an ongoing community-based cohort study of metabolic disease including type 2 diabetes and metabolic syndrome in a population aged 40 years and over. We conducted a baseline study using stratified random cluster sampling between 2003 and 2006. A total of 3,815 non-diabetic subjects (1,474 men and 2,341women) without a history of ischemic heart disease and cerebrovascular disease were followed up for an average of 4.5 years. Receiver operating characteristic (ROC) curve analysis using Youden index and the area under curve (AUC) was applied. In addition, we performed a multivariate Cox proportional hazard model adjusting age, BMI, smoking, and smoking, drinking alcohol, exercise, and dietary habits to evaluate the relative risk of development of type 2 diabetes according to the WC category for men and women.
Results: In the ROC curve analysis for the different WC cutoffs including 80 cm, 85 cm, and 90 cm, the highest value of Youden index was obtained at a WC cutoff point of 85 cm for both sexes. Sensitivity and specificity were 55.7% and 66.4% in men and 60.0% and 54.7% in women, respectively. After being controlled for other covariates, the relative risks for the development of type 2 diabetes tended to increase significantly, especially in women, as WC incremented. In addition, the relative risk for the development of type 2 diabetes using <75 cm of waist circumference as a reference increased sig-

nificantly in the category of 85-89.9 cm for women. Statistically significant associations also were consistently observed over the category of 85-89.9 cm for women.
Conclusion: The optimal cutoff value for waist circumference predicting the incident type 2 diabetes is considered to be 85 cm, especially in women, suggesting that the Asian criterion of abdominal obesity (90 cm for men and 80 cm for women) as a component of metabolic syndrome might not be applicable for middle-aged to older population in Korea.
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Association of body height with diabetes, blood pressure and metabolic syndrome among Sri Lankan adults
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Background and aims: Being tall has been suggested to be associated with better cardio-vascular health and longevity. Height, a marker of childhood growth, is associated with lower mortality and morbidity from diabetes mellitus and from associated risk factors. It is thought that better childhood conditions, such as improved nutrition and fewer respiratory infections, result in both greater adult height and lower rates of chronic non-communicable diseases. We aimed to report the relationship of height with diabetes mellitus, blood pressure (BP) and metabolic syndrome (MS) among Sri Lankan adults
Materials and methods: Data were available for height and socio-demographic factors from a nationally representative cross-sectional sample of 4477 subjects above 18 years. Recruitment was preformed between 2005-2006. Height was measured using Harpenden pocket stadiometers to the nearest 0.1 cm according to the standard methods. Subjects were considered to have 'diagnosed diabetes' if they had been previously diagnosed at a government hospital or by a registered medical practitioner. New cases ('undiagnosed diabetes') and metabolic syndrome were diagnosed according to World Health Organization criteria Seated blood pressure was recorded on two occasions after at least a 10-min rest using an Omron IA2 digital blood pressure monitor. Data were analysed using SPSS.
Results: Males were 39.5% and mean age of all subjects was 46.1 (SD±15.1) years. The mean height of all adults, males and females were 156.2±8.9cm, 163.6±6.9cm and 151.4±6.4cm respectively (p<0.001, males vs. females). In all adults Height showed a significant negative correlation with fasting blood glucose (p<0.05, r= -0.052), 2-hour post-glucose blood glucose levels (p<0.001, r = - 0.089) and diabetes (p<0.001, r = -0.069). There was a significant negative correlation between mean systolic BP and height (p<0.05, *r* = -0.032), this was not observed for the mean diastolic BP. Height demonstrated significant correlations with total cholesterol (p<0.001, *r* = -0.106), HDL cholesterol (p<0.001, *r* = -0.142), LDL cholesterol (p<0.001, *r* = -0.104) and triglyceride (p<0.001, *r* =0.064) levels. Similar changes were observed in both genders (Table 1). The mean heights of patients with MS and without MS were 154.8 ± 8.8 cm and 156.6 ± 8.9 cm respectively (p<0.001).
Conclusion: Our data showed a negative correlation between height and blood glucose levels, serum cholesterol levels and mean systolic BP. Also patients with MS were significantly shorter than those without MS. These data suggest that being tall reduces diabetes and cardiovascular risks and the underlying mechanisms needs further study.

Table:1 Relationship between height and metabolic parameters (*p<0.05)

Metabolic parameter	Correlation coefficient (*r*)		
	All	Male	Female
Fasting blood sugar	-0.052*	-0.068*	-0.052*
2 hour post glucose blood sugar	-0.089*	-0.062*	-0.089*
Presence of diabetes	-0.069*	-0.105*	-0.069*
Mean systolic blood pressure	-0.032*	-0.097*	-0.123*
Mean diastolic blood pressure	-0.028	-0.010	-0.029
Total cholesterol	-0.106*	-0.117*	-0.103*
LDL cholesterol	-0.104*	-0.102*	-0.083*
HDL cholesterol	-0.142*	-0.083*	-0.018*
Triglycerides	0.064*	0.079	-0.097*

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Neck circumference positively related with central obesity, overweight and metabolic syndrome in Chinese people with type 2 diabetes
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Background and aims: To investigate the association between neck circumference and central obesity, overweight as well as metabolic syndrome in Chinese individuals with type 2 diabetes.
Materials and methods: Subjects with type 2 diabetes (age 20-80 years) were recruited from 15 community health centers in Beijing using a multi-stage random sampling approach. Anthropometrics, including neck and waist circumference measurement was conducted. Metabolic syndrome was identified based on criteria of WHO. Central obesity was diagnosed according to criteria of IDF.
Results: 3182 diabetics (1294 men and 1888 women) without thyroid swelling, were enrolled with mean (±SD) age of 64.0±10.1 years. The diabetic duration was 9.37±6.56 years. The mean neck circumference was 36.6±3.7 cm, 38.4 ±3.6 cm for men, and 35.4±3.3 for women (p<0.001). Receiver operating characteristics (ROC) analysis showed that the area under the neck-circumference-and-central-obesity curve was 0.74 (95% C.I. (0.72, 0.77)) for men, and 0.75 (95% C.I. (0.72, 0.78)) for women (p<0.001). Furthermore, neck circumferences of ≥39 cm (with sensitivity of 56.3% and specificity of 79.2%) for men and ≥36cm (with sensitivity of 61.1% and specificity of 73.1%) for women were best cutoff levels for determining people with BMI ≥25.0 kg/m^2.Neck circumferences of ≥ 38 cm for men ,and ≥ 36 cm for women were best cutoff levels for determining people with metabolic syndrome. After adjusting with gender and age, neck circumference was associated significantly with metabolic syndrome (OR, 1.13 [95% C.I. (1.10-1.17)).
Conclusion: In the present study, neck circumference is positively related with body mass index, waist circumference and metabolic syndrome in Chinese people with type 2 diabetes. The neck circumference might be used in clinical convenient index as a predictor for central obesity and metabolic syndrome. Further studies are needed for closer identification of this association in general population.
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Impaired insulin sensitivity and beta cell function predict the onset of diabetes in non-diabetic women with former gestational diabetes
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Background and aims: Gestational diabetes mellitus (GDM) is the specific type of diabetes that may develop during pregnancy. After delivery, women with previous GDM (pGDM) often normalize their glucose levels, but they are at increased risk of developing type 2 diabetes, especially if they have other risk factors (i.e. obesity, hypertension, family history of type 2 diabetes). In this context, it is important to see whether there are indications in pGDM immediately after delivery about their possible development of overt diabetes. Aim of this study was the analysis of possible differences in insulin sensitivity and beta cell function between non-diabetic pGDM developing diabetes within 3-5 years from delivery and those who instead remained non-diabetic.
Materials and methods: A total of 77 pGDM were studied with a 75-g 3h oral glucose tolerance test (OGTT) at baseline (i.e., immediately after partum) and during the 5 year study period. Insulin sensitivity was evaluated with the oral glucose sensitivity index (OGIS), while beta cell function through mathematical modelling of C-peptide, that yields B-cell sensitivity to glucose stimulus (BGS) and early insulin response (rate sensitivity, BRS). After the baseline examination, at a following visit the OGTT of 17 women matched the ADA/WHO criteria for diabetes (progressors, PROG), while in the remaining 60 women no sign of diabetes was found (NON-PROG).
Results: In Table 1, subjects' characteristics and metabolic parameters at baseline are shown. PROG were slightly older and with higher BMI. Glucose, both fasting and during OGTT, was more elevated in PROG, while insulin was not significantly different, yielding lower insulin sensitivity (OGIS). Beta cell response was markedly impaired in PROG, especially the beta cell sensitivity to glucose (BGS) which was 40% lower.

Conclusion: Non-diabetic women with previous GDM developing diabetes within 3-5 years exhibit lower insulin sensitivity and B-cell function than pGDM who remain non-diabetic. The assessment of these metabolic parameters immediately after delivery could therefore be very useful to characterize the subjects at particularly high risk in order to start early prevention against the development of diabetes.

Table 1

	NON-PROG	PROG	p-value
Age (years)	33.4 ± 0.5	36.2 ± 1.2	0.0337
BMI (kg m^{-2})	25.6 ± 0.5	31.8 ± 1.7	<0.0001
Fasting plasma glucose (pmol l^{-1})	4.80 ± 0.06	5.45 ± 0.16	<0.0001
Plasma glucose at 2h (pmol l^{-1})	6.06 ± 0.17	8.30 ± 0.41	<0.0001
Mean plasma glucose (pmol l^{-1})	6.37 ± 0.13	8.41 ± 0.22	<0.0001
Mean plasma insulin (pmol l^{-1})	280 ± 21	336 ± 50	0.133
OGIS (ml $min^{-1}m^{-2}$)	456 ± 9	379 ± 13	<0.0001
BGS (pmol $min^{-1}m^{-2}mM^{-2}$)	106 ± 6	64 ± 7	0.0005
BRS (pmol $m^{-2}mM^{-1}$)	649 ± 66	324 ± 98	0.0321

Characteristics and metabolic parameters of the subjects at the baseline (mean ± SE).

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Contribution of different biomarkers to risk of type 2 diabetes

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Background and aims: Over the past years, a number of circulating blood biomarkers have been identified and proposed as predictors of type 2 diabetes. In the present study we quantified to what extent biomarkers of different metabolic pathways contribute to the risk of type 2 diabetes.
Materials and methods: The contribution of different biological pathways to the development of type 2 diabetes was estimated in a case-cohort design based on circulating blood biomarkers from participants aged 35 - 65 years in the EPIC-Potsdam Study. The analytic sample included 613 participants with incident diabetes and 1965 participants without diabetes. We constructed a biomarker score based on plasma values of glycated haemoglobin (HbA1c), gamma-glutamyltransferase (GGT), HDL-cholesterol, hs-CRP, and adiponectin. Cox proportional hazard regression was used to estimate relative risks adjusted for age, sex, body mass index, waist-circumference, education, sport activity, cycling, occupational activity, and smoking and alcohol intake. The proportion of the association between the score and diabetes risk explained by each biomarker was estimated using effect decomposition method by entering quintiles of glycated haemoglobin (HbA1c), gamma-glutamyltransferase (GGT), HDL-cholesterol, CRP, and adiponectin simultaneously in the model.
Results: The relative risk of type 2 diabetes between extreme quintiles of the overall biomarker index score was 14.6 (95% confidence interval (CI): 6.81, 31.2; P<0.001). A total of 27.7% (CI: 22.0, 34.1) of the risk was explained by HbA1c. For the other biomarkers the corresponding proportions were 11.5% (CI: 5.53, 17.7) by GGT, 14.4% (CI: 8.67, 20.3) by HDL-cholesterol, 6.45% (CI: -0.38, 13.4) by hs-CRP, and 13.5% (CI: 6.98, 20.3) by adiponectin.
Conclusion: The results support the hypothesis that different biological pathways reflected by markers such as HbA1c, GGT, HDL-cholesterol, and adiponectin play a role in the development of type 2 diabetes independently from each other.
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Association between liver markers and insulin sensitivity, insulin secretion and endogenous glucose production in non-diabetic individuals

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Background and aims: An association between elevated concentrations of gammaglutamyl transferase (GGT) and alanine aminotransferase (ALT) with the risk of type 2 diabetes has been shown. However, the underlying pathophysiological mechanisms remain poorly determined. The aim of our study was therefore to explore whether a modest elevation of liver markers is associated with insulin resistance and/or altered insulin secretion.
Materials and methods: We studied 1310 healthy individuals from the RISC study (Relationship between Insulin Sensitivity and Cardiovascular disease). All participants had a euglycemic-hyperinsulinemic clamp and an OGTT with determination of indices of insulin secretion. A subgroup of 409 also had an assessment of endogenous glucose production (EGP) with a tracer.
Results: Both fasting and 2 h glycaemia progressively increased over GGT and ALT quartiles. Insulin sensitivity (as assessed by the M/I value) was inversely correlated to the plasma concentration of GGT or ALT (r=-0.30, p<0.0001 for each). Modest elevations in liver markers were significantly associated with an increase risk of insulin-resistance (defined as the M/I value in the first quartile) after adjustment for age, sex, physical activity and waist: GGT>20 UI/l, OR :1.80 (1.3-2.5), p=0.0007; ALT>20 UI/l, OR :1.59 (1.2-2.2), p=0.004. There was no association between aspartate aminotransferase levels and insulin sensitivity. The hepatic insulin resistance index (EGP x fasting insulinaemia) was more strongly correlated with GGT (r=0.29, p=0.0001) than with ALT levels (r=0.16, p=0.02). These significant associations persisted in multivariate models. GGT and ALT levels were positively correlated with fasting glucagon and inversely to adiponectin concentrations. Insulin secretion, as assessed by the disposition index, was not significantly associated with liver markers. In multivariate models, GGT and ALT were independent determinant of 2h glycaemia during the OGTT.
Conclusion: GGT and ALT, even at moderately elevated levels, are closely linked to both peripheral and hepatic insulin resistance but not with insulin secretion, in healthy non-diabetic individuals. This confirms the pertinence of these markers to identify insulin resistant individuals at high risk of type 2 diabetes.
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PS 16 Novel biomarkers in diabetes prediction

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Urinary myo-inositol is a useful and cost-effective marker to detect glucose intolerance: a community-based "Tottori-Kofu" study

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Background and aims: Myo-inositol is one of 9 inositol stereoisomers with cyclic sugar alcohol and its composition is like D-glucose. Myo-inositol exists in human tissues. The urinary excretion of myo-inositol is augmented in diabetes patients, since the ascent of plasma glucose obstructs the re-absorption of myo-inositol in renal uriniferous tubule. Consequently, urinary myo-inositol excretion indirectly reflects plasma glucose level. Therefore, urinary myo-inositol could be a useful marker of glucose intolerance. We performed Tottori-Kofu study, which was designed to pick up patients with impaired glucose tolerance(IGT). We applied 75gOGTT as the most effective strategy to detect IGT. However, OGTT requires much time and cost. We presumed urinary myo-inositol could be a good candidate marker to detect IGT. In this study, we aimed to estimate urinary myo-inositol value in OGTT.
Materials and methods: We performed 75gOGTT in 110 people (male 35, female 75, average age 68.3 years old). In OGTT, we collected blood samples before, 30, 60 and 120 min. after glucose loading. Urine samples were also taken before and 120 min. after glucose loading. According to OGTT results, we segmented the group "normal glucose tolerance (NGT)" (plasma glucose <110mg/dl before glucose loading and <140 mg/dl after 120 min.), "IGT" (plasma glucose ≥140mg/dl and <200 mg/dl after 120 min.) and "diabetes mellitus(DM)" (plasma glucose ≥200mg/dl after 120 min.). We defined the subjects as pre-IGT if their plasma glucose is ≥180 mg/dl after 60 min. The OGTT results were follows: NGT 67(male15 / female52), pre-IGT 9 (m3 / f6), IGT 27 (m12 / f15) and DM 7(m5 / f2). Urinary myo-inositol(UMI) was calibrated by urinary creatinine(Ucre). We calculated ΔUMI as follows: ΔUMI(mg/gCre) =UMI/Ucre of 120min. - UMI/Ucre of 0min.
Results: ΔUMI was significantly associated with the plasma glucose response in OGTT. Estimated ΔUMI(mg/gCre) was follows; NGT(8.6±13.4), pre-IGT(26.7±18.9), IGT(27.6±30.0) and DM(97.0±48.4). The worse glucose intolerance, the higher ΔUMI would be expected. Based on the ROC curve, we set ΔUMI cut-off value as 10mg/gCre and compared the detecting ability of glucose intolerance among ΔUMI, A1C(≥6.2%) and urinary glucose excretion (≥100mg/dl after 120 min in OGTT). Within these three candidates, ΔUMI(≥10 mg/gCre) was the most powerful marker to isolate IGT. The positive rate of detecting glucose intolerance was follows; ΔUMI 69.8%, A1C 18.6% and urinary glucose excretion 55.8%.
Conclusion: We confirmed that ΔUMI derived from OGTT protocol is an effective marker to isolate glucose intolerance. We presume ΔUMI measurement could be an easy and cost-effective way in a community-based screening of glucose intolerance, since we need only urine samples, not blood, after oral glucose loading to calculate ΔUMI.

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The association between IGFBP-1 and type 2 diabetes is modified by degrees of insulin sensitivity and early insulin response

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Background and aims: Clinical studies suggest that insulin-like growth factor binding protein 1 (IGFBP-1) may be an important determinant of glucose tolerance. Further, defects in insulin secretion and action are major abnormalities in type 2 diabetes (T2DM). Accordingly, we studied the relationship between serum IGF-I, IGF-II, IGFBP-1, IGFBP-3 and T2DM, taking into account insulin sensitivity (M/I) and the early insulin response (EIR).
Materials and methods: The participants were from a population-based cohort of 71-year-old men (n=1219) from which patients taking anti-diabetic oral medication or insulin were excluded. The cohort then underwent a 7-year follow-up (n=667). At baseline a euglycaemic insulin clamp and a 75-gram OGTT were performed. The concentrations of IGF-I, IGF-II, IGFBP-1 and IGFBP-3 were measured and their respective relationship with T2DM was assessed using logistic regression, presenting odds ratios (ORs) with 95% confidence intervals (CIs) for 1 SD increase in the predictor variable, adjusted for BMI and heredity for diabetes.
Results: The cross sectional associations with T2DM are presented in Table 1. The odds ratio for the association between IGFBP-1 and a low risk of T2DM was altered after adjustment for insulin sensitivity. IGF-I, IGF-II, IGFBP-1 or IGFBP-3 were not significantly associated with the risk for T2DM over 7 years of follow-up (p=0.14-0.90).
Conclusion: The association between IGFBP-1 and risk of T2DM is modified by EIR and insulin sensitivity implying confounding effects of insulin secretion and insulin resistance on the association, whereas it was not observed for IGF-II and the carrier proteins. The IGFs and their carrier proteins did not predict T2DM but were associated with the diabetic state.

Table 1. Cross sectional associations between IGF-I, IGF-II, IGFBP-1 or IGFBP-3 and T2DM

Variable	OR, 95 %CI, crude	OR,adjusted for EIR	OR,adjusted for M/I
IGF-I	1,36, 1,07-1,73	1,53, 1,18-1,99	1,28, 0.99-1,65
IGF-II	1,33, 1,05-1,70	1,42, 1,09-1,86	1,29, 1,02-1,64
IGFBP-1	0,71, 0,56-0,90	0,52, 0,40-0,68	1,23, 0.90-1,66
IGFBP-3	1,38, 1,07-1,77	1,52, 1,15-2,01	1,34, 1,03-1,74

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The association between soluble urokinase plasminogen activator receptor (suPAR) levels and incident diabetes is modified by body weight status in high-risk people with impaired glucose regulation

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Background and aims: An association between the inflammatory marker soluble urokinase plasminogen activator receptor (suPAR) and incident type 2 diabetes mellitus (T2DM) has been documented in healthy individuals. We aimed to assess whether this association exists among high-risk people with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) and whether it is affected by other risk factors for progression to diabetes.
Materials and methods: suPAR levels were measured in plasma samples from 1933 participants of the ADDITION study (aged 37-73 years) who had isolated IFG, isolated IGT or combined IFG/IGT at baseline (2001-2006) using the suPARnostic® ELISA kit (ViroGates, Denmark). Incidence of T2DM was ascertained by OGTT at 3 years of follow-up in combination with regular reports on glucose measurements in GP practice up to December 2008. The association between suPAR levels at baseline and incident T2DM was analyzed using logistic regression. Analyses were stratified by glucose regulation status (IFG, IGT, IFG/IGT) and BMI (lean, overweight, obese - WHO definition).
Results: Adjusting for sex and age, there was a 48% overall increase in risk of developing T2DM per 2-fold increase in baseline level of fasting suPAR (Table 1). The association was modified by body weight status, being limited to overweight participants. Additional adjustment for waist, blood pressure, lipids and smoking attenuated the association between suPAR levels and T2DM in the population as a whole; however, the association in the overweight group remained robust. Glucose regulation status did not modify the association.
Conclusion: suPAR levels are associated with incident T2DM in overweight individuals with impaired glucose regulation. Our finding of an absence of association among the obese may be due to the exclusion by design of those with diabetes at baseline, or may be caused by a difference in phenotype of obese subjects compared to overweight participants. This effect needs to be examined in greater detail in future studies.

Table 1. Odds Ratios for incident type 2 diabetes per each 2-fold increase in baseline suPAR level among 1933 participants of the ADDITION study.

		sex+age adjusted	sex+age+BMI*+ waist+ bp+ triglycerides+ HDL+ smoking
	No of cases/ total	**OR (95% CI)**	**OR (95% CI)**
Whole sample	599/1933	1.48. (1.12; 1.96)	1.24. (0.91; 1.69)
By glucose regulation status**			
i-IFG	186/800	1.36 (0.84; 2.21)	1.08 (0.63; 1.84)
i-IGT	175/652	1.56 (0.95; 2.56)	1.48 (0.85; 2.58)
IFG/IGT	238/481	1.28 (0.75; 2.16)	1.12 (0.63; 2.01)
By body weight status**			
Lean (BMI<25kg/m^2)	70/341	0.65 (0.31; 1.37)	0.61 (0.26; 1.41)
Overweight (BMI ≥25 <30kg/m^2)	231/806	2.36 (1.48; 3.76)	2.14 (1.31; 2.51)
Obese (BMI≥30kg/m^2)	298/786	1.12 (0.73; 1.73)	1.00 (0.63; 1.60)

*BMI included both as linear and quadratic term; Only waist circumference was used in the models stratifying by BMI

**p for interaction = 0.93 for glucose regulation status; 0.003 for body weight status

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Leptin independently predicts diabetes in Swedish men

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Background and aims: Leptin may have detrimental effects on the beta-cell function, and may decrease insulin sensitivity. Previous studies have shown an independent association between leptin levels and future diabetes in men, but not in women, whereas other studies have opposed these findings. The aim of this study was to evaluate leptin as an independent predictor of future diabetes in a northern Sweden setting.
Materials and methods: Within the Västerbotten Intervention Program (VIP), all inhabitants were invited to a health survey the year they turned 40, 50 and 60 years old. Questionnaires, blood sampling, measurements of anthropometry and blood pressure, and an oral glucose tolerance test (OGTT) were included. Through registries covering Västerbotten County, 638 subjects with type 2 diabetes were identified, who had participated in VIP prior (more than one year, mean 6.4 years) to diagnosis. A control group of 1564 non-diabetics, group matched for age, sex, region of residence and survey date within VIP was identified. Leptin was analysed with a RIA in stored samples. Logistic regression analysis was performed, and Odds Ratios (OR) and their 95 % Confidence Interval (95% CI) calculated. Leptin data are presented as quartiles with sex-specific cut offs based on the distribution amongst controls.
Results: In the univariate analysis, high leptin (Quartile 4 vs. Quartile 1) predicted diabetes in both men (OR 8.2 95% CI 5.2-13.0) and women (OR 6.4 95% CI 3.8-10.6, p_{trend} <0.0005). After adjustments for BMI, cholesterol, smoking habits, physical activity, education level and hypertension, leptin remained associated with diabetes in men OR 2.2 95% CI 1.2-3.9, p_{trend} 0.05). In separate models adjusting for BMI and adiponectin, diabetes heredity and fasting and 2-hour glucose levels from OGTT, or for a homeostasis model assessment (HOMA 2), leptin remained associated to future diabetes in men (p_{trend} <0.0005 for all).
Conclusion: Leptin independently predicts future diabetes in Swedish men, but not in women.
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A novel method for the assessment of insulin physiology: urinary C-peptide creatinine ratio can be used to assess insulin sensitivity and beta cell function in non-diabetic subjects

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Background: Urinary C-peptide creatinine ratio (UCPCR) is a convenient, non-invasive measure of insulin secretion that is stable at room temperature for 3 days. It has been validated as a test of beta-cell function in type 1 and type 2 diabetes. The aim of our study was to test if UCPCR can be used to assess insulin sensitivity and insulin secretion in non-diabetic subjects under laboratory conditions and in the home setting.
Methods: We performed an oral glucose tolerance test (OGTT) using a standardised 75 g glucose load on 30 healthy volunteers (median age = 46 years; median BMI = 24.47). Serum insulin and C-peptide were measured on fasting blood samples and those taken at 30 min intervals for 120 min following glucose load and used to calculate insulin secretion and insulin resistance. UCPCR was measured on fasting urine samples and 120 min following glucose load. On a separate day, volunteers were asked to collect urine samples at home, 120 min following their largest meal of the day and post them to the laboratory.
Results: Fasting UCPCR correlated with HOMA-S (R=0.55; P<0.001). 120 min OGTT UCPCR correlated with serum insulin area under the curve (AUC) (R=0.69; P<0.001), serum C-peptide AUC (R=0.60; P<0.001) and early insulin secretion (R=0.41; P<0.05). Post-meal UCPCR correlated with 120 min OGTT UCPCR (R=0.55; P=0.01), serum insulin AUC (R=0.55; P<0.01), serum C-peptide AUC (R=0.53; P<0.05) and early insulin secretion (R=0.42; P=0.05).
Conclusion: In non-diabetic subjects, UCPCR measured in fasting urine samples and those collected following a glucose load or large meal are correlated with physiological measures of insulin sensitivity and insulin secretion. As urine samples are stable for 3 days at room temperature and can be collected by individuals at home, UCPCR is a convenient method for the assessment of insulin physiology that could be used in large scale epidemiological studies.
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Sialic acid predicts incident cases of hospital-treated diabetes during 40 years of follow-up in a defined population: the Värmland Health Survey, Sweden

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Background and aims: Elevated inflammatory markers correlate with features of the metabolic syndrome and predict the development of type 2 diabetes. Sialic acid (SA) is related to glycoproteins and a marker of inflammation that has previously been associated with risk of coronary heart disease (CHD) events. We examined the role of SA in predicting incident hospital-treated cases of diabetes during 40 years of follow-up.
Materials and methods: A population-based health survey was carried out between 1962 and 1965 in the county of Värmland, Sweden. In total, 47,527 men and 48,124 women participated (mean age 48.9 and 48.6 years; range 25-80 years). The screening consisted of measurement of height, weight and blood pressure, as well as blood sampling including the determination of serum SA concentration. All subjects have been followed in national registers until end of 2005 for incident cases of hospital-treated diabetes mellitus (DM) as primary or secondary diagnosis. The material was sub-divided into quintiles (Q1-Q5) from lowest (Q1) to highest SA-levels (Q5). The risk of incident DM in relation to SA was analyzed by logistic regression separately for each sex. The association between baseline SA and risk of incident hospital-treated diabetes was analyzed by Cox proportional hazards regression before and after stepwise adjustment for baseline characteristics (age, BMI, hepatic transaminase GOT).
Results: During 40 years of follow-up, in all 4591 cases of diabetes treated in hospitals was recorded for men (9.7% of all men), and 5184 cases for women (10.8% of all women). The risk (OR) of incident DM in relation to SA-

quintiles for men was 1.0 in Q1 (reference), 1.19 (95% CI 1.08-1.30) in Q2, 1.20 (1.09-1.32) in Q3, 1.13 (1.02-1.25) in Q4 and 1.12 (1.02-1.24) in Q5. In women the corresponding OR was 1.0 in Q1, 1.24 (CI 1.12-1.36) in Q2, 1.31 (1.19-1.43) in Q3, 1.56 (1.42-1.71) in Q4 and 1.56 (1.45-1.74) in Q5. After full adjustment in the Cox-regressions, Exp (B) for one standard deviation of SA was 1.11 (95% CI 1.08-1.14) in men. The corresponding risk for women was 1.14 (1.11-1.17). P-values for all analyses were <0.0001.

Conclusion: The risk of incident diabetes mellitus in relation to high levels of sialic acid as an inflammatory marker is stronger in women than in men. The increased risk was independent of the other baseline characteristics.

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Cardiovascular risk factors and micro- and macrovascular complications in patients with type 2 diabetes in Italy

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Background and aims: Type 2 diabetes mellitus (T2DM) is associated with increased morbidity and mortality for cardiovascular disease (CVD) and microvascular complications. This study aimed at assessing the prevalence of CVD risk factors and micro and macrovacular complications in patients with T2DM in Italy.

Materials and methods: We used baseline data from the Renal Insufficiency And Cardiovascular Events (RIACE) Italian Multicenter Study, a prospective cohort study on reduced estimated GFR as an independent predictor of CVD morbidity and mortality in T2DM subjects. The RIACE cohort consists of 15,773 T2DM patients consecutively visiting 19 Diabetes Clinics throughout Italy in years 2007-2008. Exclusion criteria were dialysis or renal transplantation. The following information was collected by a structured interview: age, gender, smoking status, diabetes duration, current therapy, and previous major acute CVD events. Height, weight, blood pressure (BP), HbA_{1c}, triglycerides (TG), total- and HDL- cholesterol (C), creatinine and albuminuria were measured, GFR was estimated using the 4-variable MDRD equation, and retinopathy was assessed with ophtalmoscopy or retinography

Results: Age was 66.0± 10.3 years, diabetes duration 13.2± 10.2 years, and male (M)/female (F) ratio 57/43. HbA_{1c} was 7.55±1.51%, with 24.2% and 40.9% below 6.5% and 7%, respectively; 61.4%% were on oral agents, 15.5% on insulin, and 9.6% on combined therapy. BMI was 28.96±5.14, with 41.9% overweight, and 24.4%. 8.4% and 3.3%, respectively, with grade I, II and III obesity. Waist was 103.7±12.7 cm, with 51.8% M below 102 and only 12.4% F below 88. TG, HDL-, LDL- and non-HDL-C levels were 139.2±88.2, 49.8±13.6, 107.7±32.6 e 134.9±36.8 mg/dl, respectively, with 68.3% on-target for TG, 50.7% of M and 56.8% of F for HDL-C, 42.1% for LDL-C and 48.3% for non-HDL-C; 46.2% were on lipid-lowering drugs and 42.5% on a statin. BP levels were 138.1±18.0 and 78.8±9.4 mmHg, with 43.5% and 73.1% with systolic and diastolic values <130 and 80 mmHg, respectively; 70.7% were on anti-hypertensive agents and 58.1% on RAS blockers. Current, former and never smokers were 15.3%, 28.1% and 55.6%, respectively. History of any CVD event was detected in 23.2%, myocardial infarction in 11.1%, stroke in 3.3%, foot ulcer/gangrene in 3.4%, and coronary, carotid and lower limb revascularization in 10.0%, 4.9% and 2.9%, respectively. Background and advanced retinopathy were detected in 12.5% and 9.6%, and micro and macroalbuminuria in 22.2% and 4.7%, respectively. Prevalence of GFR classes 1, 2, 3 and 4-5 as estimated by the MDRD formula was 29.6%, 51.7%, 17.1% and 1.6%, respectively. Of the 2,960 patients with chronic kidney disease, 56.6% were normo, 30.8% micro and 12.6% macroalbuminuric.

Conclusion: Results of this large-cohort study indicate a relatively good control of CDV risk factors and low prevalence of complications in Italian T2DM patients.

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Diabetes duration: a crucial factor when determining individual target levels of glycaemic control in old age? (ZODIAC-20)

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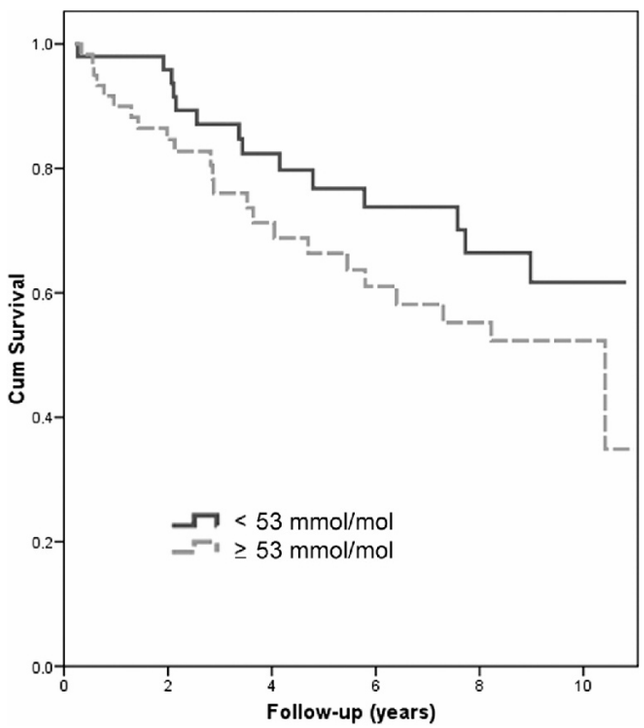
Background and aims: Studies regarding macrovascular consequences of glucose control in elderly patients (>75 years) with type 2 diabetes mellitus (T2DM) are lacking. This study aimed to investigate the relationship between HbA1c and (cardiovascular) mortality, and the role of diabetes duration in this relationship, in an elderly population.
Materials and methods: Between 1998 en 1999, 374 primary care patients older than 75 years with T2DM participated in the ZODIAC study, a prospective observational study. Data on mortality were collected in 2009. A Cox proportional hazard model was used to investigate the relationship between HbA1c as a continuous variable, as well as dichotomous variables (HbA1c <53 mmol/mol vs. HbA1c ≥53 mmol/mol, and HbA1c <48 mmol/mol vs. HbA1c ≥48 mmol/mol) and mortality. Analyses were performed in strata according to diabetes duration (<5 years, 5-11 years, and ≥11 years). Age, gender, smoking (yes or no), BMI, duration of diabetes, serum creatinine level, macrovascular complications (yes or no), albuminuria (yes or no), systolic blood pressure, total cholesterol-HDL ratio, and use of insulin (yes or no) were selected as possible confounders.
Results: In the group with a diabetes duration <5 years, the hazard ratios for HbA1c as a continuous variable were 1.40 (95%CI 1.14-1.73) and 1.66 (95%CI 1.25-2.21) for all-cause and cardiovascular mortality respectively. Within this patient group, a HbA1c level of <53 mmol/mol at baseline was associated with a reduced all-cause and cardiovascular mortality risk compared to patients with worse glycemic control. The hazard ratios were 0.56 (95%CI 0.33-0.95) and 0.28 (95%CI 0.11-0.69) respectively. Figure 1 illustrates the increased cardiovascular mortality rates for patients with a HbA1c level ≥53 mmol/mol. Glycemic control was not related to mortality for patients with a diabetes duration ≥5 years.
Conclusion: Worse glycemic control is related to increased mortality in elderly diabetic patients, but only in those with T2DM of short duration. Randomised controlled trials are necessary to answer the question whether improving glycemic control is beneficial in elderly patients. Perhaps different optimum target levels should be used for T2DM of short and long duration.



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The role of cardiovascular risk factors in postmenopausal hypercholesterolemic women with abnormal fasting glucose: a post hoc analysis of the MEGA Study

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Background and aims: The incidence of cardiovascular disease (CVD) is increased in postmenopausal women, and the presence of CVD risk factors diminishes the favorable profile of women against CVD. Thus, understanding the role of CVD risk factors for CVD events and identifying effective interventions is important in postmenopausal women. The aim of this study is to assess the combined effect of CVD risk factors including abnormal fasting glucose (AFG) on the development of CVD in hypercholesterolemic postmenopausal women, and to evaluate the effect of low-dose pravastatin treatment, using the data from a large-scale clinical trial (MEGA Study).
Material and methods: The MEGA Study examined the effect of low-dose pravastatin (10-20 mg/day, approved dose in Japan) on primary prevention of CVD in 7,832 Japanese patients (5,356 women, post-menopause to 70 years; 2,476 men, 40 to 70 years) with mild to moderate hypercholesterolemia. Patients were randomized to diet alone (3,966 patients) or diet + pravastatin (3,866 patients) and followed for an average 5 years. AFG was defined as fasting plasma glucose (FPG) ≥6.1 mmol/L or anti-hyperglycemic agent users. Patients not having FPG value were excluded from this analysis (n=1,661). Normal fasting glucose (NFG) was defined as FPG <6.1mmol/L. Hypertension was defined by the attending physician as ≥140/90 mmHg, using the Japan Society of Hypertension guideline. The incidence of CVD in relation to age, hypertension, and AFG were compared between sexes. Hazard ratios (HRs) for incident CVD were calculated by using Cox's multivariable proportional hazards model, with the combinations of age, hypertension, and AFG, adjusted for treatment arm, high density lipoprotein cholesterol, and smoking.
Results: The incidence of CVD events was 2.3% (125/5,356) in women and 5.3% (130/2,476) in men. Table shows HRs for incident CVD for the eight possible combinations of the analyzed risk factors (age, hypertension, AFG). In women, compared to men, the risk for CVD was higher for each possible combination of age, hypertension and AFG, although no sex interaction was found for any risk factor combination due to small number of events. A similar risk reduction for coronary heart disease and cerebral infarction was found in the diet + pravastatin group compared to diet alone group (23% for women, p=0.39; 43% for men; p=0.03; interaction p=0.81).
Conclusion: The combining the older age and hypertension in AFG markedly increased the CVD risk in postmenopausal hypercholesterolemic women than men. Women with AFG may achieve a similar risk reduction for coronary heart disease and cerebral infarction with low-dose pravastatin as did men with AFG.

HRs for incident CVD for the combinations of age, hypertension, and AFG

Age ≥60y	HT	AFG	Men			Women		
			n. (Events/ Patients)	HRs	*P*-values	n. (Events/ Patients)	HRs	*P*-values
-	-	-	9/536	1.0		4/955	1.0	
+	-	-	8/191	2.6	0.0142	12/857	3.3	0.0391
-	+	-	12/258	3.0	0.0496	12/512	4.9	0.0059
-	-	+	14/299	2.7	0.0207	4/291	3.0	0.1241
-	+	+	17/183	5.4	<0.0001	9/207	8.8	0.0003
+	+	-	13/190	4.6	0.0005	27/723	8.3	0.0001
+	-	+	17/177	5.9	<0.0001	18/303	13.5	<0.0001
+	+	+	22/152	9.4	<0.0001	21/337	12.7	<0.0001

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Peptic ulcers and inflammatory diseases of the upper digestive tract increase the risk of incident diabetes independently of obesity. The Whitehall II study

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Background and aims: Recent interest in the effect of intestinal microflora and gastric bypass surgery on diabetes risk has highlighted the close connection between the gastro-intestinal (GI) system and glucose metabolism. We studied whether a diagnosis of upper GI peptic/inflammatory or ulcerative disease increased the risk of subsequent diabetes during up to 20 years of follow up.
Materials and methods: We studied 6766 participants of the Whitehall II study [69.7% men, mean age: 49.9 years (SD:6.0), BMI: 25.3 (3.7)] free of diabetes and GI diagnoses at baseline in 1991-1993. Participants were followed for incident diabetes with repeated OGTTs at 5-year intervals and self reports at 2.5-year intervals until December 2009. Hospital diagnoses were obtained from Hospital Episode Statistics data, covering all admissions to National Health Service hospitals in England. Participants with oesophagitis, gastritis, duodenitis, dyspepsia, gastro-oesophageal reflux or peptic ulcers of the oesophagus, stomach or duodenum (ICD-10 codes K20-K22, K25-K27, K29-K31) prior to diagnosis of diabetes or end of follow up were considered exposed. Their risk of incident diabetes was compared to the risk in those not exposed to an upper GI diagnosis using multivariate Poisson regression adjusting for relevant confounders.
Results: During a mean follow up of 16.9 years (total: 114,442 py), we observed 772 cases of incident diabetes (incidence rate: 8.0 per 1000 py) and 363 upper GI diagnoses. Mean time between GI diagnosis and DM was 5.5 years (SD: 3.3). The risk of diabetes increased after an upper GI diagnosis (Table 1). Adjustment for BMI, waist circumference, HDL cholesterol, triglycerides, fasting glucose and hs-CRP did not attenuate the effect. Models assuming that exposure starts with a 1-year lag after an upper GI diagnosis showed very similar results. The proportion of diabetes cases diagnosed through a study visit OGTT did not differ significantly between those with and without a GI diagnosis, indicating that detection bias is unlikely. Results were also consistent when examining oesophageal or gastric/duodenal diagnoses separately.
Conclusion: We found that peptic/inflammatory or ulcerative conditions in the upper GI tract increase the risk of diabetes by 10% per year in subsequent years independently of central and over-all obesity as well as other major risk factors for diabetes. Although baseline low grade inflammation did not explain our results, a later inflammatory response may play a role, in conjunction with alterations of the internal environment in the upper GI tract. Our results indicate that clinicians should be alert to the elevated risk of diabetes in patients with upper GI peptic/ulcerative diseases.

Table 1. Incidence rate ratio's (IRR) for the risk of diabetes after an upper GI diagnosis

Per year after diagnosis		
	IRR	(95% Confidence Interval)
Model A	1.08	(1.02;1.15)*
Model B	1.09	(1.03;1.16)*
Model C	1.10	(1.04;1.17)*
'Ever diagnosed'		
	IRR	(95% Confidence Interval)
Model A	1.69	(1.23;2.32)*
Model B	1.74	(1.27;2.40)*
Model C	1.85	(1.35;2.55)*

Adjustments: Model A: age and sex, Model B: A + BMI and waist, Model C: B + HDL-cholesterol, triglycerides, fasting glucose and hs-CRP. *: p-value <0.01.

Supported by: Funding structure: http://www.ucl.ac.uk/whitehallII/index.htm

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Increased risk of acute renal failure in patients with type 2 diabetes compared to those without diabetes

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Background and aims: Acute renal failure (ARF) is characterized by a rapid decline in glomerular filtration rate and can occur in patients with normal renal function as well as patients with pre-existing renal disease. ARF is a broad condition encompassing a spectrum of clinical renal dysfunction. While the natural history of a progressive decline in renal function from diabetic nephropathy has been well-described in patients with type 2 diabetes mellitus (T2DM), few studies have assessed the risk of ARF in a large population of T2DM patients. This study quantified the risk of ARF associated with T2DM in a large general practice database from the United Kingdom. In addition, the risk of ARF in T2DM patients with multiple comorbidities was assessed.
Materials and methods: Patients with T2DM (n=148,963) based on diagnosis, prescriptions or laboratory glucose and patients without diabetes (n=2,834,927) were identified from the General Practice Research Database. Patients with end-stage renal disease were excluded from the study. Crude incidence and age/gender-adjusted hazard ratios (HR) of ARF were estimated for T2DM and non-DM. Multivariate Cox regression models adjusted for risk factors including prior ARF, chronic kidney disease (CKD - including diabetic nephropathy), congestive heart failure (CHF), hypertension (HT), alcohol use, obesity, smoking, and Charlson comorbidity index. To assess potential additive effects, ARF risk was also assessed in patients with T2DM plus CHF and/or HT relative to patients without T2DM.
Results: Between 2003 and 2007, ARF incidence was 192 per 100,000 person-years (p-y) in patients with T2DM compared to 24/100,000 p-y among patients without T2DM (crude HR 8.3, 95% CI 7.7, 8.9). Age, obesity, prior ARF, CHF, CKD, HT, and comorbidity index were also higher in patients with T2DM. The risk of ARF for T2DM patients remained significant but attenuated in multivariate analyses adjusting for these factors (adjusted HR 2.4, 95% CI 2.2, 2.5). Adjusted ARF risk was also increased for patients with CHF (adjusted HR 2.2, 95% CI 2.0, 2.4), HT (adjusted HR 2.0, 95% CI 1.8, 2.2), and all of the above factors except CKD. Interestingly, the presence of CKD was not associated with an increased risk of ARF diagnosis (adjusted HR 1.25, 95% CI: 0.97 - 1.59). Adjusted ARF risk increased in T2DM patients with CHF and/or HT- T2DM with no HT/CHF: HR 2.3 (95% CI 2.1, 2.5), T2DM+HT (no CHF): HR 2.0 (95% CI 1.8, 2.3), T2DM+CHF (no HT): HR 4.5 (95% CI 3.7, 5.5), T2DM+HT+CHF: HR 3.5 (95% CI 2.7, 4.5).
Conclusion: Patients with T2DM have increased risk for ARF compared to patients without diabetes, even after adjustment for known risk factors including CKD. The finding that CKD alone is not associated with an increased risk of ARF diagnosis needs to be confirmed in other studies. The combination of T2DM, CHF, and HT further increased the risk for ARF relative to patients without T2DM. Physicians should be aware of this increased risk of ARF in T2DM patients, and the additional risk associated with the presence of other comorbidities such as CHF and HT.

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Oral contraceptive use and abnormal glucose regulation in Swedish middle aged women

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Background and aims: Use of oral contraception has been suggested to increase the risk of type 2 diabetes. Results of previous studies are however conflicting. The aim of this study was to investigate the association between oral contraceptives (OCs) use and abnormal glucose regulation in Swedish middle aged women.
Materials and methods: The present study includes 4794 women, aged 36-56 at baseline (1996-98), residing in five municipalities in Stockholm County Council and participating in the cross-sectional and follow-up study of Stockholm Diabetes Prevention Programme, which is a prospective population-based study. At both baseline and follow up 8 years later, the women were examined by oral glucose tolerance test (OGTT) classifying the subjects as having normal glucose tolerance, prediabetes (impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or IFG+IGT) or type 2 diabetes. In addition, anthropometric measurements were collected and a questionnaire

was answered, including questions on lifestyle factors and use of OCs in different doses of estradiol and progesterone.
Results: In the cross sectional study, current use of OCs was associated with prediabetes, odds ratio (OR)=4.1 (95%CI: 2.2-7.8) but not with type 2 diabetes. The association to prediabetes was entirely linked to IGT, OR=7.1 (3.3-15.8) in current users of OCs and in former users, OR=2.1 (1.1-3.9). Women who used OC at baseline had a better cardiovascular risk profile with lower BMI, were more physically active and smoking was less common. At the follow up, the increased risk for prediabetes did not persist.
Conclusion: Current use of OC was associated with a four times increased risk of having prediabetes and seven times increased risk of having impaired glucose tolerance. No increased risk persisted at the follow-up, suggesting that the risk of prediabetes due to prior use of OC is decreasing with time. The healthier lifestyle in women who used OCs may have contributed to reduce the long-term risk of prediabetes.

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Diabetic patient or patients with diabetes: an approach from comorbidity in primary health care

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Background and aims: To describe differences in burden of illness and variability in the Family Physicians management of patients with diabetes depending on their co-morbidity.
Materials and methods: Cross-sectional observational and descriptive study. Participans and settings: 129 family physicians selected by quality clinical records, attending 149417 patients during 2007. Computerized clinical record and pharmaceutical billing database: after grouping all patients with an episode of diabetes through case-mix ACGs System, the following indicators were built: chronic co-morbidities, consumption of visits, pharmaceutical consumption and referrals and standardized morbidity rate (SMR, using standard prevalence observed in all patients). To analyse variability of family physicians management of diabetes, two indicators were calculated for each family physician: Risk Index (RI) that relates the complexity of patients with the standard (if > 1, more complexity) and Efficiency Index (EI) that reports on the management of patients relating the consumption observed with expected, based on attended case-mix (if > 1, more consumption than expected). Variability indicators: coefficient of variation (CV), Extremal Quotient (EQ), excluding the outliers beyond of the 5-95 percentiles (EQ5-95) and of the 25-75 percentiles (EQ25-75)
Results: 10058 (6.7%) patients suffering from diabetes with an mean age of 66.7 years (SD 15.1), 52.6% were female. 9.7% of those with diabetes has no associates chronic co-morbidities and 41% had 3 or 4 and 34% 5 and more chronic co-morbidities. Most frequent pathologies were hypertension (61.2%), dyslipidaemia (44.5%), obesity (33,05%) and depression (21.5%). The correlation between the burden of illness and the number of visits, pharmacy and the number of referrals was R=0.99 (R^2=0.99), R=0, 99 (R^2=0.99) and R=0.97(R^2=0,94) respectively. SMR was higher than 1 for most of chronic diseases , especially hepatic chronic disease, skin ulcer, kidney chronic disease, chronic heart failure and coronary heart disease; and was lower than 1 for osteoporosis. Variability indicators using visits criterion was RI range: 0.7-1.2 (CV = 9% EQ5-95=1.4; EQ25-75=1.1) and EI range: 0.3-1.8 (CV=21% RV5-95=1.8; RV25-75=1.3) and using pharmaceutical consumption criterion RI range: 0.5-1.3 (CV=18%; EQ5-95=1.9; EQ25-75=1.3) and EI range: 0.9-3.3 (CV= 3% RV5-95=1.9; RV25-75=1.3).The correlation between RI and EI concerning visits consumption is R=-0.21 (R^2=-0.05) and pharmaceutical consumption R=-0.36 (R^2=0.13)
Conclusion: Most of diabetic patients has high associated co-morbidity, especially hypertension, dyslipidaemia, obesity and depression. Burden of illness seems to highly determine the impact of diabetic patients consumption on the National Health System. There is important variability in the family physician management of diabetic patients no related with complexity of case-mix.
Supported by: Fondo de Investigación Sanitaria, Instituto de Salud Carlos III

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Prevalence of diabetes mellitus and pre-diabetes in an Irish cohort age 45-75 years

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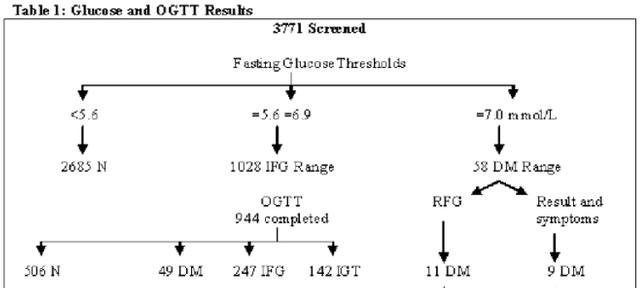
Backgrounds and aims: Type 2 diabetes (T2DM) and pre diabetes are preventable if known risk factors are identified and modified. To date there have been no large-scale studies of the prevalence of T2DM or pre-diabetes in Ireland. To address this issue the largest Irish health insurer, (covering over 62% of individuals with Private Medical insurance; 1.4 million members), has undertaken a study of the prevalence of undiagnosed T2DM and pre diabetes by screening 30,000 members. We report results from the first year of screening. To identify the prevalence of undiagnosed T2DM, impaired fasting glucose (IFG), impaired glucose intolerance (IGT) and diabetes risk in an Irish population.
Materials and methods: Members without a diagnosis of T2DM, aged 45 to 75 years living within 5 km of two major University Teaching Hospitals in Dublin, were chosen randomly and invited to participate. Participants completed a detailed medical questionnaire. Fasting plasma glucose (FPG), lipid profiles, blood pressure, weight, height, BMI, waist circumference, and waist: hip ratio were measured. Those subjects with initial FPG results of greater than or equal to 5.6 less than or equal to 6.9mmol/L had an Oral Glucose Tolerance Test (OGTT) performed. Those with an FPG greater than or equal to 7.0mmol/L had a repeat FPG performed. The Diabetes Risk Score was calculated based on FINRISK.
Results: 3771 participants (2125 female / 1646 male), were screened. Mean Age 60 ± 8.3 years (59.8 years ±8.2 for women; 60.2 years ± 8.3 for men). In the total screened group: T2DM = 1.8%, IFG = 6.5%, IGT = 3.8%. However among those subjects with an FPG between 5.6-6.9 mmol/L: T2DM = 5%, IFG = 26%, IGT =15 %. Glucose and OGTT results are summarised in the Table 1. Diabetes Risk Score results indicated that over 25 % of participants had a moderate or greater risk of developing T2DM (15 % women; 10 % men).
Conclusion: The prevalence of undiagnosed T2DM in the population screened to date is low, however over 10% of participants have pre-diabetes. Performing an OGTT as follow up on FPG 5.6-6.9 mmol/ identified either T2DM or pre diabetes in 46% of those tested. In conclusion screening for T2DM or pre diabetes is achievable in this setting, identifies unrecognised vascular and diabetes risk and could lead to disease prevention if the identified risk factors are modified.

Table 1: Glucose and OGTT Results



OGTT Results	Total Population	IFG Range: Initial F. Gluc. =5.6 =6.9	Initial Fasting Gluc. =5.6 =6.0	OGTTs Results (944)
DM	1.3%	5.0%	DM	1.6%
IFG	6.5%	26%	IFG	15%
IGT	3.8%	15%	IGT	8%
N	13%	54%	N	46%

N = Normal; RFG = repeat fasting glucose; F = Fasting; Gluc = Glucose

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Health-related quality of life: a marker of increased mortality in elderly type 2 diabetic patients (ZODIAC-18)

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Background and aims: Diabetes-related complications and physical impairment are more prevalent in old age, and are likely to have detrimental effects on health-related quality of life (HRQOL). The aim of the present study was to assess the relationship between HRQOL and (cardiovascular) mortality, and the role of age in this relationship.
Materials and methods: Between 1998 en 1999, 1353 primary care patients with type 2 diabetes mellitus (T2DM) participated in the ZODIAC study, a prospective observational study. Early 2009, data on mortality were collected. HRQOL at baseline was assessed using the RAND-36 questionnaire. A Cox proportional hazard model was used to investigate the relationship between HRQOL and mortality. Analyses were performed in strata according to age: ≤ 75 years (n=979) and >75 years (n=374). The following variables were selected as possible confounders: age, gender, smoking (yes or no), systolic blood pressure, BMI, duration of diabetes, macrovascular complications (yes or no), albumin-creatinine ratio, serum creatinine level, total cholesterol-HDL ratio, and HbA1c.
Results: After a follow-up time of 10 years, 570 out of 1353 patients (42%) had died, of whom 280 deaths (41%) were attributable to cardiovascular causes. The Physical Component Summary (PCS) in both age groups was inversely related to all-cause and cardiovascular mortality (table 1; the hazard ratios refer to an increase of 10 points in the HRQOL scores). For the Mental Component Summary (MCS) an inverse relationship was observed only for patients aged 75 years of younger. Two separate RAND-36 dimensions were inversely related to all-cause and cardiovascular mortality in both age groups: physical functioning and general health perception.
Conclusion: Decreased HRQOL was related to increased all-cause and cardiovascular mortality in both younger and older patients with T2DM. These results are of special interest for elderly patients, since traditional risk factors become less predictive of mortality at higher age. HRQOL may be a useful tool to identify elderly patients at high-risk of mortality.

Hazard ratios of all-cause and cardiovascular mortality for PCS and MCS

RAND-36	All-cause mortality	Cardiovasular mortality
Physical Component Summary		
≤ 75 years	0.86 (0.80-0.93)	0.83 (0.73-0.93)
> 75 years	0.89 (0.81-0.96)	0.87 (0.76-0.98)
Mental Component Summary		
≤ 75 years	0.88 (0.81-0.95)	0.84 (0.74-0.95)
> 75 years	0.92 (0.85-1.00)	0.96 (0.84-1.08)

PS 18 Diabetes comorbidities: hospitalisation and cancer

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Analysis of hospital admission causes in diabetic patients

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Background and aims: To describe the clinical features of diabetic patients who require hospital admission and the main reasons.
Materials and methods: Descriptive cross-sectional, observational study. Participants and settings: 584896 persons assigned to one of the Primary Health Care Centres attached to a reference hospital. From Primary Health Centres and hospital medical records, clinical data, number of admission and diagnostics that motivated during 2007 were registered. Hospitalization episodes were pooled using the patients DRGs (diagnostic related groups) classification system.
Results: 26845 patients were diabetics (4.6%), of which 10.6% (2840) had at least one admission. 51.6% of admitted were women and 52.2% of those who never was admitted during 2007, p = 0. 56. Admitted diabetic patients generated 4032 episodes of hospitalization, 1.4 admissions/patient (range 1-14). 61% was an emergency admission and the reason for discharge was recovery in 98.4%. Hospital death rate was 1.2%. The most common ICD-9 codes were coronary atherosclerosis 2.5%, congestive heart failure 2.0%, leg degenerative osteoarthritis 1.8% and intermediate coronary syndrome 1.6%. The admissions obtained by major diagnosis categories (MDC) distribution was 23.6% circulatory, 9.9% musculoskeletal, 9.6% respiratory and 9.2% digestive pathology. Episodes concerning endocrine, nutritional and metabolic were 4.6% of the cases. DRGs associated to diabetes appear only in 2.6% of hospitalisations. More frequent DRGs were respiratory disorders (3.4%) excluding infections, bronchitis and asthma complications or major co-morbidity, followed by two related heart failure DRGs (4.7%) Avoidable hospitalizations according to list of validated Ambulatory Care Sensitive Conditions (ACSC) for Spain diabetes codes were only 39 cases (0.97%) of this admissions group.
Conclusion: Cardiovascular pathology is the most usual cause of hospitalization in diabetic patients. Other complications of diabetes do not appear to be frequent admission reasons for patients with diabetes. A limited number of hospital admissions of diabetics are avoidable, which may reflect an appropriate management of diabetics in Primary Health Care.
Supported by: Fondo de Investigación Sanitaria, Instituto de Salud Carlos III

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The impact of diabetes mellitus in a tertiary hospital

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Background and aims: The number of people with diabetes worldwide is set to double in the next 20 years. The impact of diabetes mellitus and associated co-morbidities on a tertiary Hospital is not completely understood. The aim of this study is to characterize the diabetic population admitted to our Hospital and analyze its evolution in the years 2000, 2005 and 2009.
Materials and methods: A retrospective, descriptive and correlational study, encompassing patients with diabetes (as primary or secondary diagnoses) admitted to a tertiary Hospital in the years 2000, 2005 and 2009 was performed. We evaluated age, gender, type of diabetes, cause of admission, length of stay, macrovascular complications, such as acute myocardial infarction and stroke and in-hospital mortality. Our results were compared with a national diabetes database**.** We used the International Classification of Diseases-9-Clinical Modification and the information provided by the local Medical Support System database. Statistical analysis of the data was performed using Statistical Package for Social Sciences 18. Inferential statistics (t test, ANOVA and X^2)was used to conduct an analysis of age, length of stay and in-hospital mortality predictors.
Results: The total patients admitted to our Hospital in these three years was 85588, 24635 in the year 2000, 30261 in the year 2005 and 30692 in the year 2009. In these years, 872 patients (4%), 1874 patients (6%) and 2205 patients (7%) had the diagnosis of diabetes, as primary or secondary, diagnoses in a total of 4951 diabetic patients (6%), 2624 (53%) male. The rate of diabetes as

a primary diagnosis was 14,3% and among these 30,6% were type 1 diabetes. We included patients of all ages (9 months - 100 years) with a mean of 69±16 years (2000: 68±16 years; 2005: 69±16 years; 2009: 70±16 years). In patients with type 1 diabetes the average age was 39±28 years and in patients with type 2 diabetes 69±15 years (p<0,001). Acute myocardial infarction was the primary diagnosis in 353 diabetic patients (7,1%) and stroke in 91 patients (1,8%). The average length of in-hospital stay of diabetic patients in the three years was 10±12 days. The average length of stay of type 1 diabetic patients was lower comparing with patients with type 2 diabetes (p=0,019). Age (p<0,001) and macrovascular complications (p<0,001) were also predictors of increased length of stay. Comparing the average hospitalization time between diabetic and non-diabetic patients, there was no significant difference (p=0,202) in year 2000. Inversely, there were significant differences in the years 2005 (p<0,001) and 2009 (p<0,001). The in-hospital mortality rate in diabetic patients was three times higher than that of non-diabetic patients (12% versus 4%, p<0,001). Age (p<0,001), diabetes as secondary diagnosis (p=0,013) and macrovascular complications (p=0,004), were predictors of higher mortality.

Conclusion:

1- The number of hospitalizations and the average age of diabetic patients, as primary or secondary diagnosis, have increased over a ten year period.

2- Patients with type 2 diabetes have a longer length of stay than patients with type 1 diabetes, probably related to their advanced age.

3- The average length of stay and in-hospital mortality in diabetic patients were higher comparing with national diabetes database.

4- This study suggests the need for a more aggressive intervention in the prevention, diagnosis and treatment of diabetes mellitus, reinforcing the need for optimal metabolic control for prevention of macrovascular complications.

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Gender effect on the relation between diabetes and hospitalisation for heart failure

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Background and aims: Heart failure (HF) is a major cause for hospitalization especially in the elderly, and, at the same time, is mainly due to ischemic heart disease which, as well known, is strongly related to diabetes mellitus. It is likewise known that women are more protected than men toward the risk of cardiovascular diseases at least during the premenopausal period, while diabetes fully reverses such advantage, conferring to postmenopausal females a higher risk for cardiovascular complications and consequently HF. With such premises one can expect that gender difference is able to modify the association between diabetes and HF hospitalization, but no clear information so far exists about this point. More details on this point might be obtained by the availability of a wide centralized database containing all discharges across a given period of time from hospitals of a homogeneous region.

Materials and methods: To achieve this purpose we used a database concerning all hospital discharges from the wards of internal medicine (n=362,352M and n=385,806F) and of cardiology (n=91,411M and 49,212F) in Tuscany, Italy (3,686,377inhabitants) during the period 2002-2007 with a DRG derived from the corresponding ICD-9-CM codes of diabetes (250.xx) and/or of HF (DRG 127, containing ICD-9-CM codes 401.91, 402.01, 402.11, 402.91, 404.01, 404.3, 404.13, 404.93, 428.0, 428.1, 428.9) as main or secondary diagnosis. We considered discharges above age of 30 years due to the very low prevalence of HF below this age and calculated the relative risk and 95% CIs of being diagnosed diabetic in patients hospitalized for HF, stratified for age and sex.

Results: HF related hospitalization rate increased with age from 2.3% in the age group 30-39yr to 27.3% in the age group ≥90yr in men and from 1.1% to 30% in females, while diagnosis of diabetes progressively increased from the first decade to a maximum in age class 70-79yr (16.4% in men and 18.1% in females) and then decreased with further ageing in both sexes. The relative risk (95% CI) of HF hospitalization was about two-fold higher in diabetic than in non diabetic individuals across the entire observation period [1.97 (1.34-3.41) in males and 2.16 (1.60-3.53) in females]. The association diabetes-HF had a 'horse-shoe' pattern significantly raising in decade 30-39yr [2.27 (1.56-4.03)] (only in males), then reaching a maximum in class 40-49yr for males [3.24 (2.64-3.53)] and in decade 50-59yr for women [3.75 (1.94-4.03)], and afterwards progressively decreased, remaining more elevated in females during the entire period 40-69yr, and becoming equal in both genders during the following decades.

Conclusion: Hospitalization risk for HF was about two-fold higher in patients of both sexes with diabetes, being more elevated in females than in males in the life period ranging between 40 and 60 years, thus disclosing a risk peak linked to the existence of a 'perimenopausal effect'.

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Glucose status in emergency medical admissions to one of the five busiest emergency hospitals in the Republic of Ireland

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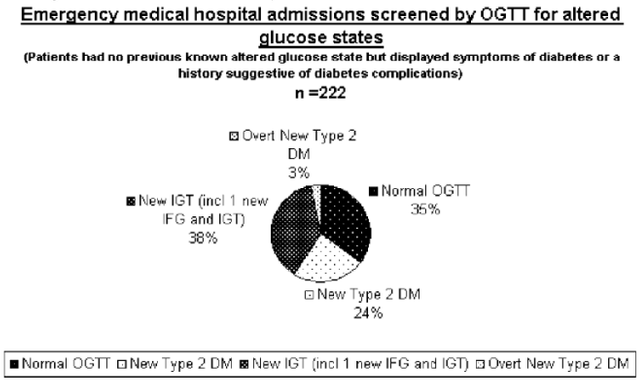
Background and aims: Decreasing physical activity and increasing obesity of ageing populations in Western Europe contribute to increased incidence of impaired glucose tolerance (IGT) and diabetes mellitus (DM). Stress hyperglycaemia is common in acutely ill patients. Dysregulation of glucose is associated with increased morbidity / mortality and has major financial implications for healthcare. In this study, glycaemic status was determined in emergency medical admissions to the one of the five busiest emergency hospitals in the Republic of Ireland.

Materials and methods: During an 18 month time frame, an unselected 1237 (14%) of all 8659 emergency medical admissions were enrolled in the study. Patients with prior diagnosis of diabetes, impaired fasting glucose (IFG) or IGT were recorded. In the remaining patients, a 75g OGTT was performed on patients (18 years or older) who displayed symptoms of diabetes or a history suggestive of diabetes complications. OGTT glycaemic status was recorded according to WHO 2006 definitions.

Results: 270 of the sample admissions had previously diagnosed type 1, type 2 diabetes, or IGT. 6 new cases of type 1 diabetes were diagnosed at the time of admission without the need for OGTT. 719 sample admissions (58%) did not meet the inclusion criteria for performing the OGTT. 20 of the sample admissions were excluded from the OGTT due to age <18 years or known endocrine disease. OGTT was performed in the remaining 222 (18%) of sample admissions. All 222 were Caucasian with median age 71 years, IQ range 58 years to 81 years, 122 (55%) male. 55% were admitted due to cardiological illness, 12% due to neurological illness (including stroke or transient ischaemic attack) and 12% due to respiratory illness. 78 (35%) of OGTT screened patients were demonstrated to be normoglycaemic. 84 (38%) of OGTT screened patients demonstrated IGT and 53 (24%) demonstrated FPG or 2h-PG in the diabetic range. 144 (65%) of OGTT screened patients had abnormal glucose states that were previously undiagnosed representing 12% of the admissions sample. Adding to the 270 (22%) of admissions who had previously known abnormal glucose states, we demonstrated a minimum of 33.5% of emergency medical admissions that had abnormal glucose states.

Conclusion: This study indicates that 65% of patients admitted to one of the five busiest emergency admissions hospital of Ireland with symptoms of diabetes or its complications but not previously diagnosed with glucose dysregulation had impaired glucose tolerance or diabetes diagnosed by a OGTT during their stay. Whether this persists after their recovery needs to be ascertained, however there is still a considerable burden on diabetes services during their acute admission stay.



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Elevated basal insulin increases the risk for total mortality in cancer incident subjects: The Israel Glucose Intolerance, Obesity and Hypertension 25-year Follow-Up Study

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Background and aims: Type 2 diabetes has been associated with increased incidence, in the range of 1.2-2.5, for cancers of the liver, pancreas, endometrium, breast, colon, and bladder, and non-Hodgkin's lymphoma. Possible upstream factors, i.e. shared risk factors of diabetes and cancer, such as obesity, hyperlipidemia, and baseline hyperinsulinemia, have been suggested as explanations. The aim of this study was to investigate endogenous insulin as a risk factor in 25-year cumulative cancer incidence and as a prognostic factor among subjects who developed cancer.
Materials and methods: We followed cancer incidence and mortality in a sample of 1770 non-diabetic men and women, with mean age at baseline of 52.1+8.0, from 1980 until 2005. Baseline fasting, 1 and 2-hour post-load plasma glucose and insulin levels were recorded. All subjects were free of cancer at baseline. Cancer incidents occurring within the first 2 years of follow-up were excluded.
Results: During the follow-up period 327 individuals (18.5%) developed cancer (3.0%, 2.2%, and 2.7% developed breast, prostate, and colon/rectum cancers, respectively). Fasting insulin was not found to be significantly associated with overall cancer incidence, or with incidence of specific sites (breast, prostate, colon/rectum, or bladder). Median survival time for cancer patients in the upper fasting insulin quartile at baseline (>18.9 mU/L) was half that of those in the lower three quartiles: 4 years, 95%CI = 2-13 years, compared to 8 years, 95%CI = 6-15 years; p=0.1. In a Cox Proportional Hazard model, adjusting for age, sex, and ethnic origin, fasting insulin in the upper quartile conferred a 53% increased risk for total mortality (95%CI = 1.04 - 2.23) compared to the lower quartiles. Male sex, age, and ethnic origin were also found to be associated with a greater risk for mortality (p = 0.003, < 0.001, 0.03 respectively).
Conclusion: This long term cohort study suggests a role for elevated insulin levels in adulthood, not as a carcinogen, but as a factor adversely affecting cancer prognosis.

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Diabetes is associated with an increased risk of cancer in the Canarian population

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Background and aims: While obesity has shown an association with most types of cancer, the association of diabetes with the disease is still controversial. The Canary Islands (Spain) have a high prevalence of obesity (32% of the adult population), diabetes (13%) and the metabolic syndrome (25.3%, according to the criteria of the International Diabetes Federation). Furthermore, some types of cancer are also increased in our population, especially breast cancer. The aim of this study was to assess the relationship between diabetes and the risk of cancer in the Canarian population.
Methods: A cross-sectional study was performed on the subjects enrolled in the "CDC (cancer, diabetes and cardiovascular disease) of the Canary Islands", a population-based cohort of 6729 individuals, aged 18-75 years, chosen randomly from the census of health care system affiliates (covering 99% of the population). Recruitment took place between 2000 and 2005. Clinical information was obtained using a questionnaire, anthropometric measurements were taken and blood samples were drawn. Diabetes was defined if the patient had a known diagnosis and/or received glucose-lowering treatment. In the absence of information confirming the diagnosis, diabetes was defined by fasting glucose concentrations above 125 mg/dl on two separate occasions. Cancer was self-referred and confirmed with data obtained about hospital admissions and treatment prescriptions. The risk of cancer was compared between 6721 subjects (8 were lost due to absence of reply) with and without diabetes (chi-squared, Yates' continuity correction). In order to adjust for possible confounders, a multiple logistic regression analysis was performed, including the overall risk of cancer as the dependent variable and age, gender, body mass index, years of smoking and parental history of cancer as independent variables. A p value below 0.05 was considered significant.
Results: Information was available for analysis from 6721 subjects of the cohort. The overall prevalence of cancer was 1.8%: 3.9% among the diabetic and 1.5% among the non-diabetic subjects (OR 2.604 [95% CI 1.694-4.002], p<0.0005). After adjustment for possible confounders, the association between diabetes and cancer remained significant (OR 1.633 [1.034-2.579], p=0.036). Gender (OR 0.511 [0.327-0.797], p=0.004 for males), age (OR 1.067 [1.048-1.086] per year, p<0.0005) and maternal history of cancer (OR 2.391 [1.598-3.579], p<0.0005) were also associated with cancer in this model, whereas years of smoking, body mass index and paternal history of cancer were not.
Conclusions: Diabetes is associated with an increased risk of cancer in the Canarian population, independently of age, gender, body mass index, smoking and parental history of cancer.
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Diabetes and cancer in Mauritius

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Background and aims: Although evidence supporting the association of diabetes with cancer risk is growing, there are no data on diabetes and cancer in South Asian and Africans populations. The aim of this work was to explore the relationship between diabetes, impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) and all-cause and site-specific cancer mortality adjusted for conventional risk factors in a large multi-ethnic cohort living in the developing nation of Mauritius.
Materials and methods: Population-based surveys were undertaken in Mauritius in 1987, 1992 and 1998 (n= 9559). Participants were aged 18 years or greater and 66%, 27%, and 7% were of South Asian (Indian), African (Creole), and Chinese descent, respectively. Response rates for all surveys were greater than 85%. Questionnaires, anthropometric measurements, and a 2 hour 75-g oral glucose tolerance test were undertaken at each survey. Glucose tolerance was classified according to World Health Organisation 1999 criteria. In 2007, a mortality follow-up was undertaken. Cox's proportional hazards model with age as the time scale was used to obtain hazard ratios (HRs) for risk of all-cause cancer and site-specific cancer mortality, adjusted for sex, smoking, prior CVD, ethnicity, hypertension, education, waist and hip circumference, and leisure time physical activity.
Results: Over a median follow-up of 15.1 years, there were 1559 deaths (199 due to cancer), 7168 survivors and 832 participants were lost to follow-up. After exclusion of deaths in the first year after baseline, compared with those with normal glucose tolerance (NGT), the all-cancer mortality HRs (95% confidence intervals (CIs)) for known diabetes mellitus (KDM), newly diagnosed diabetes mellitus (NDM), impaired glucose tolerance and impaired fasting glycaemia were 2.34 (1.19 to 4.59), 1.80 (1.00 to 3.26), 1.34 (0.73 to 2.46) and 0.80 (0.28 to 2.25) in men and 1.19 (0.63 to 2.22), 0.58 (0.26 to 1.33), 1.34 (0.80 to 2.27) and 0.53 (0.13 to 2.22) in women, respectively. In men, but not women, total cancer mortality risk increased with rising plasma levels of postload glucose - HR for the top versus bottom quintile was 2.52 (1.34 to 5.59), Ptrend <0.025 in fully adjusted models. There was no relationship between fasting plasma glucose and total cancer mortality in either sex. The HRs for breast and reproductive tract cancer mortality for women with diabetes (KDM and NDM) compared to women without diabetes were 2.67 (0.95 to 7.67) and 1.09 (0.28 to 4.26) in South Asians and Africans, respectively after adjustment for age, smoking and waist circumference.
Conclusion: This is the first study in a developing country of the impact of glucose intolerance on cancer mortality in an African or South Asian population. The independent association of diabetes (KDM and NDM) and post-

load glucose with total cancer mortality risk in men in this study provides first time evidence of such a relationship in Africans and South Asians. These results are important in a global context for future health policy in the light of the impact of the rapid increase in prevalence of diabetes and cancer, especially in developing nations.

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Chronic hepatitis B infection increased risk of all site cancer in type 2 diabetic patients with suboptimal glycaemic control

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Background and aims: Over 10% of people in China have chronic HBV infection and type 2 diabetes (T2DM). Both of these chronic conditions have been implicated in carcinogenesis. We hypothesized that chronic HBV infection may interact with poor glycaemic control to increase risk of cancer in type 2 diabetes.
Materials and methods: In a prospective cohort from the Hong Kong Diabetes Registry (HKDR) consisting of 4205 T2D patients with known HBV status and detailed documentation for risk factors, complications, treatments and clinical outcomes followed up for a mean period of 6 years, we examined the incidence of cancer in patients stratified by A1c and explored their interactions with HBV carrier status. Cox proportional hazard regression was used to obtain hazard ratios (HRs) of HBV infection for cancer in univariable models and multivariable models. To test possible interactions between HBV infection and hyperglycaemia for cancer, we separately performed initial checking of the HRs of HBV infection for the risk of cancer among patients with A1c<7.4% and among those patients with A1c≥7.4% in univariate and multivariate models. We further examined biological interaction of HBV infection and hyperglycaemia (i.e., A1c≥7.4%) using: 1) Relative excess risk due to interaction (RERI); 2) Attributable proportion due to interaction (AP); and 3) Synergy index (S).
Results: HBV carriers have increased risk of cancer (HR 1.70; CI: 1.28-2.25, p<0.0001). When divided according to HbA1c, the hazard ratios of all-site cancer was 2.23 (CI: 1.57-3.16; p<0.0001), after adjustment of other clinical attributes. Interactive models suggest the increased risk of cancer in HBV carriers is present among subjects with HbA1c ≥7.4%. Analysis using the RERI, AP or S index revealed there was statistically significant biological interaction between HBV carrier status and HbA1c ≥ 7.4 mmol/L for the risk of cancer in Type 2 diabetes.
Conclusion: In type 2 diabetic patients with suboptimal glycemic control, the coexistence of chronic HBV infection substantially increased the risk of cancer. The rapid transition from communicable to noncommunicable diseases in China will have major implications on morbidity and mortality including cancer.
Supported by: HKFRDD, LWY Diabetes Research Fund

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Risk factors for pancreatic cancer aetiology of glucose intolerance

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Background and aims: Relationship between the incidence of pancreatic cancer and the development of diabetes is unclear. Diabetes has been suggested to be a risk factor for pancreatic cancer. On the other hand, glucose intolerance or frank diabetes is often the first sign of a pancreatic cancer, a fatal disease unless diagnosed at a very early stage. However, at present with diabetes being so prevalent, cancer etiology of glucose intolerance might be easily missed, bringing fatal consequences upon a patient. We studied glucose tolerance in patients with newly diagnosed pancreatic cancer aiming at identifying factors suggestive of cancer etiology of glucose metabolism disturbances. The additional aim of the study was to assess endothelial dysfunction and subclinical inflammation as markers of cardiovascular risk, often neglected in this group of patients.
Materials and methods: The study group was 18 non-diabetes individuals with newly diagnosed pancreatic cancer (PC group) (mean age 69.6±8.9 years, BMI 23.0±4.7 kg/m2); 13 age- and body weight-matched healthy subjects served as controls. All subjects underwent oral glucose tolerance test (OGTT) according to WHO protocol with plasma glucose and insulin measurements. HOMA index and fasting plasma adiponectin, TNF-alfa, interleukin-6 (IL-6), interleukin-1beta (IL-1b), sE-selectin, thrombomodulin, soluble adhesion molecules sICAM and sVCAM, and high-sensitive CRP were assessed.
Results: PC and control subjects plasma glucose values in OGTT were at 0 min 98±21 and 90±17, 60 min - 166±43 and 103±36 (p<0.01), 120 min - 173±39 and 90±28 (p<0.001) mg/dl, and plasma insulin at 0 min 3.6±1.8 and 10.2±7.9 (p<0.01), 60 min - 22.3±17.4 and 33.1±21.4, 120 min - 31.9±19.3 and 34.1±44.7 mIU/l. PC patients presented also with significantly greater insulin sensitivity as measured with HOMA than the controls (0.83±0.39 vs 2.34±1.87; p<0.01). Moreover, PC subjects as compared with the controls had significantly greater plasma adiponectin (16132±8165 vs 6681±4329 ng/ml; p<0.001), IL-6 (8.6±4.9 vs 3.9±1.4 pg/ml; p<0.01), thrombomodulin (2.0±0.8 vs 1.1±0.5 ng/ml; p<0.01), sICAM (929±417 vs 318±85 ng/ml; p<0.001) and sVCAM (1669±489 vs 857±312 ng/ml; p<0.001).
Conclusion: Individuals with newly diagnosed pancreatic cancer present with elevated post-challenge plasma glucose associated with significant insulin sensitivity. Diagnosing glucose metabolism disturbances in lean subjects with high insulin sensitivity should point at the possibility of pancreatic cancer as an underlying condition. Moreover, despite absence of insulin resistance, newly diagnosed PC subjects present with clinical markers of subclinical inflammation and endothelial dysfunction.

PS 19 Early mechanisms in autoimmune diabetes - animal models

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An experimental study showing dietary gluten as triggers of type 1 diabetes

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Background and aims: Type 1 diabetes (T1D) is a multifactorial autoimmune disease and besides genetic susceptibility several environmental factors such as diets and gut microbiota contribute to the onset of the disease. Recent epidemiological data indicate that T1D incidence in Europe might double around the year 2020. Genetic factors cannot explain this dramatic increase, which indicate that changes in environmental factors are mainly responsible. There is accumulating evidence that dietary gluten might be important dietary triggers of T1D. Epidemiological data show that early introduction of dietary gluten increases the chance to develop T1D. Also aberrant immune responses against wheat gluten have been found in T1D patients. In this study we investigated in the diabetes prone (DP)BB rat animal model for T1D whether dietary gluten can modulate diabetes development and potential mechanisms involved such as changes in gut microbiota composition, intestinal barrier function and (innate/mucosal) immune status.
Materials and methods: BBDP rats were fed specific diets with different amounts of gluten. One group received the hydrolysed casein (HC) diet. The HC-diet is gluten free and the proteins are degraded to small fragments leading to the loss of diabetogenic epitopes. The other groups received the HC-diet with 2%, 4% and 10% gluten respectively. The control group received the standard diet with ±4% gluten. Rats were followed for T1D development and feces, gut tissue, lymph-nodes and blood were collected. Because gluten can affect intestinal permeability, BBDP rats were subjected to a Lactulose Mannitol (LA/MA) test to measure intestinal permeability in vivo. Composition of gut microbiota was established in the feces by qPCR.
Results: Addition of 10% gluten to the HC-diet completely abrogated the diabetes protective effect of the HC-diet. HC-fed rats (n=21), 50% diabetic median age 85 days vs HC+gluten fed rats (n=14), 90% diabetic median age 76 days ($p<0.05$, log rank test). Moreover, addition of 10% gluten also completely reversed the HC-diet induced improved intestinal barrier function as shown by a 2-fold increased urinary LA/MA levels ($p<0.05$, MWU test) and a trend for a reduced ileal expression of claudin-1 mRNA ($p<0.1$, MWU test). We have shown previously that the HC-diet lowers intestinal *bacteroides* levels of BBDP rats. Yet, the gut microbiota composition of HC fed rats did not differ from HC+gluten fed rats. High levels of anti gliadin antibodies were found in sera of HC+gluten fed rats, indicating systemic immune activation. Effects of gluten on (innate/mucosal) immune status are now under investigation.
Conclusion: Gluten are potent dietary triggers of T1D. Manipulating intestinal barrier function and (innate/mucosal) immune activation might be important mechanisms for this effect. Better insight in how dietary gluten can induce T1D development will lead to dietary intervention strategies in order to reduce the chance to develop T1D in people at risk.
Supported by: Dutch Diabetes Foundation

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Rapid influx of macrophages into peritoneal cavity of NOD mice after weaning suggests increased permeability to gut bacteria

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Background and aims: Increased gut permeability has been proposed as an important mechanism of sensitization against T1D-related autoantigens in gut immune system. The distal part of gut also contains a large ecosystem of microbes, which are important in creating the right balance of helper-T-cell subsets for gut immune responses. Our recent findings indicate an excess of Th17 cells and other inflammatory cells in colons of young NOD mice. The aims of this study were to investigate if this inflammation leads to extra-intestinal manifestations such as increased peritoneal cell numbers, and any signs of bacterial leakage from the gut.
Materials and methods: Peritoneal leukocytes in NOD and BALB/c mice were counted and phenotyped using trypan blue, flow cytometry and real-time PCR. Presence of bacteria was investigated with PCR using primers for bacterial 16S-RNA. IL-1β, IL-6 and IL-12 were analyzed using qt-PCR.
Results: In peritoneum of 4,5 week-old NOD mice total number of macrophages was 3.0(±0.43)x10E6 compared to 0.76(±0.23)x10E6 in BALB/c mice and 0.68(±0.51)x10E6 in NOD mice fed antidiabetogenic Prosobee-diet ($p < 0,001$, one-way ANOVA). At the same age, bacterial 16S-RNA PCR revealed the presence of bacterial DNA in peritoneum of 3/20 NOD mice compared to 0/20 BALB/c mice (N.S.). In all 3 cases, sequencing identified the presence of the same bacterial species in NOD peritoneum. The excess of macrophages did not associate with increased production of proinflammatory cytokines in the peritoneum compared to BALB/c mice.
Conclusion: Shortly after weaning, there is a rapid "influx" of macrophages into peritoneum of NOD mice which is prevented by antidiabetogenic diet. Simultaneously, bacteria may gain access to extra-intestinal locations. This may stimulate the innate arm of systemic immune responses and break tolerance to autoantigens.
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Daintain/AIF-1 plays important role in the initiation and progress of type 1 diabetes

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Background and aims: Type 1 diabetes (T1D) is characterised by progressive destruction of pancreatic beta cells as a consequence of infiltration by mononuclear cells and lymphocytes. The early stage of the disease process is termed insulitis. The beta cell death in the course of insulitis is suggested to be caused by direct contact with activated macrophages and T-cells, and/or exposure to soluble mediators secreted by these cells, including cytokines, nitric oxide (NO), and oxygen free radicals. The molecular mechanisms for the pathogenesis of T1D are still incompletely understood. Daintain/AIF-1, as an inflammatory factor, is secreted by macrophages and T cells. We have previously demonstrated a densely accumulative daintain/AIF-1 immunostaining in the pancreas of BB rats, an animal model of T1D, when they were suffering from insulitis. The aim of the present study was to further investigate the roles of daintain/AIF-1 in the pathogenesis of T1D.
Materials and methods: Studies were performed in NOD mice, an animal model of T1D, using immunoprecipitation, Western blots, immunohistochemistry, Total Internal Reflection Fluorescence Microscope (TIRFM), flow cytometry, and affinity chromatography.
Results: Using immunoprecipitation followed by Western blot, we found that daintain/AIF-1, as a circulating protein, exists in the blood of non-diabetic Balb/c and diabetic NOD mice, but the plasma concentration in NOD mice was distinctly higher than in age-matched Balb/c mice (40 ug/L vs 10 ug/L, $p < 0.001$), which suggests that daintain/AIF-1 associates with T1D. We also found that daintain/AIF-1 immunostaining appeared in the pancreas of NOD mice when they already suffered with T1D, and no insulin was detected in the pancreas of those animals, indicating that daintain/AIF-1 may be involved in beta cell death in T1D. When daintain/AIF-1 was intravenously injected into NOD mice (100ug/25 g body weight, 3 times in other 10 days), the white blood cell proliferation in the mice was largely increased (4.5×10^9/L vs 1.7×10^9/L, $p < 0.001$), indicating that daintain/AIF-1 is able to promote inflammation in the mice. In parallel, the plasma concentration of insulin was gradually decreased and the plasma glucose concomitantly increased. Finally, using immunocytochemistry it was also found that the beta cells were undetected, but the alpha cells existed in the pancreas, which suggests that daintain/AIF-1 selectively damage beta cells. Using TIRFM and flow cytometry, we found Daintain/AIF-1 is able to infiltrate into the primary pancreas beta cells and damage the cells *in vitro*. With affinity chromatography and followed by peptide mass fingerprinting, a kind of Daintain/AIF-1-interacting proteins was identified as cystathionine beta-synthase (CBS) from the mice pancreas. CBS is involved in the metabolism of homocysteine, the later is a risk factor for damaging tissues, and beta cells are very sensitive to homocysteine.
Conclusion: Taken together, these results indicate that daintain/AIF-1 plays important roles in the initiation and progress of T1D. Daintain/AIF-1 will be a novel target molecule for prevention and treatment of T1D.
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Previous Coxsackievirus B3 infection in mothers protect offspring from virus-induced type 1 diabetes

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Background and aims: Type 1 diabetes (T1D) results from the destruction of the pancreatic insulin-producing beta cells. It is a complex autoimmune disease involving both genetic and environmental risk factors. Epidemiological studies and clinical observations point to an association between T1D and enterovirus infections, in particular those with Coxsackie B virus serotypes (CVBs); Enteroviruses have been identified in and isolated from the pancreas of newly diagnosed T1D patients. Moreover, enterovirus RNA has been more frequently found in serum from recent-onset T1D patients than in serum from healthy controls. Despite the strong association between enterovirus infections and T1D, recent studies in different human populations (countries) have suggested that the presence of enterovirus infections in the background population shows an inverse relationship with the population incidence of T1D. This counterintuitive finding has lead to the suggestion that enterovirus infections may have a more severe impact in populations where infections occurr less frequently (a.k.a. the poliovirus hypothesis). Indeed, low background herd immunity to a pathogen may lead to more severe disease in infected individuals. This may in part be due to a reduced transfer of maternal antibodies to offspring (passive immunity). To date there has been no attempts to experimentally test this hypothesis. Thus, the aim of this study was to establish a model in which we could determine whether maternally transferred antibodies protect genetically susceptible offspring from enterovirus-induced T1D.
Materials and methods: The present study used the SOCS-1 transgenic NOD model (SOCS-1 Tg NOD) that develops T1D rapidly after infection with CVB3 and CVB4, and non-tg NOD mice that do not develop T1D upon infection with these viruses. NOD females were infected intra-peritoneally (i.p.) with an immunizing dose of CVB3 (10^2 pfu). After the acute infection was cleared, infected and uninfected females were bred with SOCS-1-Tg NOD males, progeny was challenged i.p. with a diabetogenic dose of CVB3 (10^3 pfu). Diabetes development was monitored by daily blood glucose measurements. Neutralizing antibodies were quantified by a serum neutralization assay.
Results: Immunized NOD females (n=3) had CVB3-neutralizing antibodies in the serum (p<0,001, vs. uninfected controls, n=4). The majority of SOCS-1-transgenic offspring from uninfected mothers developed T1D after infection (83%, n=10/12). In contrast, only approximately half (54%, n=13/24) of the transgenic offspring from immunized mothers developed T1D (p=0,14).
Conclusion: Preliminary observations suggest that passive immunization via the transfer of antibodies from the mother protects offspring from virus-induced T1D. These results support the extended poliovirus hypothesis as an explanation for the reported inverse correlation between the observed number of enterovirus infections and the incidence of T1D in a population. These studies may also have important implications for the development of an effective therapy to protect neonates from enterovirus infections. Our findings indicate that vaccination of women before childbirth could be an efficient therapy to prevent enterovirus infections in progeny, and thereby possibly also virus-induced T1D.
Supported by: the Swedish Diabetes Research Association and the Swedish Child Diabetes Foundations

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Apoptosis resistance of non-obese diabetic mouse thymocytes is mediated by a defective p53 expression and altered microRNA network controlling cell death and proliferation

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Clonal deletion during negative selection in the thymus is fundamental in order to establish central tolerance and to avoid the development of autoimmune disorders such as type 1 diabetes (T1D). It has been previously shown that NOD thymocytes display a defect in the establishment of central tolerance as well as and resistance to cell death when exposed to apoptosis inducing agents. However, the underlying mechanisms contributing to these traits are still elusive. The tumour suppressor gene p53 has been demonstrated to be involved in thymocyte apoptosis and is up-regulated in response to DNA damage and during thymocyte selection. Our aim was to study the molecular basis of apoptosis resistance of NOD thymocytes and to understand whether this process could be involved in diabetes pathogenesis in NOD mice. Here we were able to show that NOD mice are unable to up-regulate p53 and caspases 1 and 11 after γ-irradiation compared to wild type C57BL/6 mice. Recently, it has been shown that p53 is regulated by the microRNA (miRNA) family miR-34. We found a decreased expression of mir-34a and mir-34b/c in NOD thymocytes in response to DNA damage. Sequencing of the miR-34 gene family revealed 3 significant polymorphisms in the miR-34a gene in NOD mice. Interestingly, one of the nucleotide variant in NOD miR-34a gene is located in close proximity to a p53 binding site. The miR-34a gene is located in the mouse *Idd-9.2* diabetes susceptibility locus, and the miR-34b/c genes are clustered in the *Idd2*-locus. Thus, the miR-34 genes represent potential candidate genes for diabetes development in NOD mice. In conclusion, our results highlight the molecular basis for thymocytes apoptosis resistance observed in NOD mice, which might ultimately predispose to the development of autoimmune diabetes.
Supported by: VR

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MafA is a key regulator of insulin expression in the thymus

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Background and aims: Tissue-specific self-antigens are ectopically expressed within the thymus and play an important role in the induction of central tolerance. Abnormal regulation of the intra-thymic expression of a certain self-antigen is therefore expected to cause organ-specific autoimmune disease. Insulin is expressed in both pancreatic islets and the thymus, and is considered to be the primary antigen for type 1 diabetes. Here, we report the role of insulin transactivator MafA in the expression of insulin in the thymus and susceptibility to type 1 diabetes.
Materials and methods: The expression profiles of transcriptional factors (*Pdx1*, *NeuroD*, *Mafa* and *Aire*) in pancreatic islets and the thymus were examined in NOD (non obese diabetic) and control mice(C3H mice). The nucleotide sequence of *Mafa* was identified by direct sequencing in NOD, C3H, Balb/c, CTS and NSY mice. Luciferase reporter assay was performed for newly identified promoter polymorphisms of NOD *Mafa* sequences .
Results: *Mafa*, *Ins2* and *Aire* expression was detected in the thymus. *Mafa* expression was significantly lower in NOD thymus than control, and was correlated with *Ins2* expression(R^2=0.809). When the data were stratified by strain, the correlation was stronger in control (R^2=0.863) than in NOD mice (R^2=0.393). No correlation was found between *Ins2* and *Aire* in either control C3H (R^2=0.145) or NOD mice (R^2=0.135). The entire nucleotide sequence of mouse *Mafa* was identical among the control strains (Balb/c, C3H and CTS mice) and an animal model of type 2 diabetes (NSY mice). Only NOD mice showed variation in the nucleotide sequence of *Mafa* compared to other strains. NOD *Mafa* promoter activity was significantly lower than that of wild type by 27% (p<0.0001).
Conclusion: These data suggest that MafA is a key regulator of insulin expression in the thymus, and functional polymorphisms of *Mafa* are newly identified in the NOD mouse.
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Altered leukocyte recruitment during inflammation in type 1 and type 2 diabetes models

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Background and aims: Diabetes is often associated with a systemic low-grade inflammation, the origin of which is uncertain. Despite the low-grade inflammation, diabetic patients are known to have an impaired bacterial clearance. To fight an infection, leukocytes need to leave the circulation at the afflicted

site, which occurs through regulated steps of activation and interactions of endothelium and leukocytes. The effect of hyperglycemia on leukocyte function is a matter of debate. The aim of this study was to evaluate leukocyte recruitment in established models of inflammation during high- and moderate hyperglycemia.
Materials and methods: The experimental models of diabetes used in the current study were induced either by i.v. administration of alloxan, causing a severe hyperglycemia, or by a high fat diet, causing a moderate hyperglycemia. In anaesthetized diabetic or control C57Bl/6 mice, the number of adherent and emigrated leukocytes was studied using intravital microscopy of the exposed cremaster muscle before and during exposure to the chemokine MIP-2. Bacterial clearance in alloxan- or untreated mice was studied after a subcutaneous injection of luminescent *S. aureus* using a non-invasive IVIS camera system, and the fraction of recruited leukocyte subtypes was analyzed at different time points post infection.
Results: During basal conditions, prior addition of chemokines, both severe and moderate hyperglycemia resulted in increased numbers of adherent and emigrated leukocytes compared to normoglycemic mice. In response to MIP-2 addition, the number of emigrated cells was significantly increased in severely hyperglycemic mice compared to control mice. However, alloxan-treated mice demonstrated decreased number of recruited leukocytes after 5 and 10 days post infection and an impaired ability to clear bacterial infections compared to control mice.
Conclusion: In conclusion, in both alloxan-, and high fat diet-induced diabetes, leukocyte recruitment was increased in untreated as well as chemokine exposed cremaster muscles, indicating that acute hyperglycemia activated leukocytes and/or endothelial cells. However, the ability to clear bacterial infections was reduced in alloxan-treated mice due to fewer amounts of recruited leukocytes later in the recruitment process.
Supported by: Thurings stiftelse

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Metabolic phenotypes in preclinical autoimmune diabetes in man and mouse: pathways behind progression to overt disease
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Background and aims: The incidence of type 1 diabetes among children and adolescents has increased markedly in the Western countries during the recent decades and is presently, for unknown reasons, increasing at a faster rate than ever before. Recent evidence from serum metabolomics indicates that specific characteristic metabolic disturbances precede β-cell autoimmunity markers and accurately identify those children who subsequently progress to diabetes. The causes and tissue-specific mechanisms leading to these early metabolic disturbances are unknown.
Materials and methods: A total of 70 mice (26 female) were monitored weekly with serum collection from age 3 weeks until either (a) the development of diabetes and then followed by treatment with insulin for 4 weeks (progressor group), or (b) followed until 36 weeks of age in females and 40 weeks in males in the absence of a diabetic phenoptype (non-progressor group). Global lipidomics using UPLC/MS was applied on all 1172 samples, and a biomarker for diabetes prediction in 8 week old female mice was developed based on insulin autoantibody (IAA) level and the lipidomic profile. Computational statistical modeling was applied to compare and align the longitudinal lipidomic profiles of NOD mice with the profiles of children who later developed diabetes. In an independent experiment normoglycemic female NOD mice were sacrificed at 8 (n=57) or 19 (n=14) weeks of age and blood, liver and pancreas samples were collected. The mice were stratified according to high or low risk of developing diabetes based on lipidomics-derived serum marker. Additionally, lipidomics, metabolomics (GCxGC-TOFMS) and transcriptomics were applied to isolated islets and liver.
Results: The specificity of the pre-autoimmune metabolic changes identified in serum lipidomes of human type 1 diabetes progressors, as indicated by their conservation in murine model of type 1 diabetes. We show that young female non-obese prediabetic mice who later progress to autoimmune diabetes exhibit the same lipidomic pattern than prediabetic children, and that these changes are accompanied by dysregulation of specific metabolic and immunoregulatory pathways in pancreatic islets and liver. Specifically, plasma insulin, insulinotropic amino acids in islets, and pathways of liver insulin signaling were already significantly upregulated in those prediabetic mice at high risk of developing diabetes. The pathogenic relevance of these metabolic pathways is further suggested by the observation that insulin autoantibody positive mice, that were protected from diabetes, had reduced mitochondrial pathways including OXPHOS in the islets.
Conclusion: The findings indicate that autoimmune diabetes is preceded by a state of islet increased metabolic demands resulting in elevated insulin secretion and suggest alternative metabolic related pathways as potential therapeutic candidate targets.
Supported by: EU FP7 project DIAPREPP

PS 20 Intervention in animal models of type 1 diabetes

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Depletion of IL-2/15 receptor beta-positive cells protects from diabetes in non-obese diabetic mice: role of natural killer cells and a subset of CD8+ T cells

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Background and aims: A role for natural killer (NK) cells in type 1 diabetes has been suggested, but the literature is conflicting. We have recently shown NK cells with unique phenotypic and functional properties in the pancreas of NOD mice. This project aims at elucidating a possible role of NK cells in type 1 diabetes (T1D) by applying in vivo depletion of NK cells in non-obese diabetic (NOD) mice using the monoclonal antibody TMβ1.
Materials and methods: TMβ1 is a monoclonal antibody directed against the beta chain of the murine IL-2/15 receptor (CD122). CD122 is highly expressed on all NK cells and on a small subpopulation of CD8+ T cells in the spleen. 200ug of the antibody to CD122 (TMβ1) was given i.p. once weekly to NOD mice and urine glucose measured. The effect on NK cells and T cell subsets were investigated in the pancreas, spleen and draining pancreatic lymph nodes (PLN). In order to investigate the presence and phenotype of pancreatic lymphocytes without contamination of lymphocytes from blood or lymph nodes the mice were flushed with PBS via the heart and lymph nodes removed under microscopy. The effect of TMβ1 treatment on lymphocyte infiltration into the pancreas was also explored using immunohistochemistry. NK cell function after TMβ1 treatment was assessed in vivo as the elimination of fluorescently labelled MHC class I-deficient spleen cells. The influence of TMβ1 treatment on primary CD8+ T cell responses was studied as IFNg production following immunization with the non diabetogenic antigen GP33. The TMβ1 effect on priming in the draining lymph nodes was investigated in an adoptive transfer model where BDC2.4 transgenic CD4+ T cells bearing a diabetogenic TCR were labelled with cells labelled with the fluorescent dye CFSE and transferred to NOD mice treated with TMβ1 or PBS. CFSE distributes equally in divided cells, and was used to measure proliferation after 3 days.
Results: In vivo administration of the TMβ1 antibody to non-obese diabetic (NOD) mice protected against diabetes development when administered just before disease onset. Depletion removed all NK cells and most CD122-expressing CD8+ T cells in the spleen. Some CD8+ T cells in the pancreas also disappeared despite lack of CD122 expression, suggesting either an indirect effect of NK cells, or reduced trafficking of CD122+ peripheral T cells to the pancreas. NK cell function is totally abolished after TMβ1 treatment whereas specific CD8+ T cell responses remain unaltered and the levels of the activation marker CD69 is unchanged on T cells both in the pancreas, spleen and PLN. Preliminary results suggest that TMβ1 treatment does also not affect the proliferation of T cells in the PLN. The possibility that the cytokine production may be altered in these proliferating T cells is being investigated. A detailed immunohistochemical analysis of the infiltrating lymphocytes is also currently ongoing.
Conclusion: We propose that targeting of CD122+ cells may represent a novel treatment strategy against late events in type 1 diabetes. With only minor effects on the immune system this treatment could prolong the disease free period, or even prevent disease onset, in patients at risk of developing type 1 diabetes.
Supported by: the Swedish Diabetes Association

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Efficient gene transfer to the pancreas using adeno-associated viral vectors for prevention of type 1 diabetes in mice

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Background and aims: Gene therapy may provide new treatments for severe pancreatic disorders. However, gene transfer to the pancreas is difficult because its anatomic location and structure, and pancreatitis is a serious concern. We have previously shown that pancreatic beta cells can be transduced to express marker genes after systemic injection of first generation adenovirus (FGAd) in mice with clamped hepatic circulation. However, FGAd are not suitable for long term effects because the immune system recognizes and eliminates transduced cells due to the expression of viral genes. Here we investigated whether adeno-associated viral vectors (AAV), which lack all viral genes, are low immunogenic and capable of long-term expression, were able to mediate efficient gene transfer to the pancreas.
Materials and methods: AAV serotypes 6, 8 and 9 carrying GFP as a marker gene or murine hepatocyte growth factor (mHGF) were generated by triple transfection in 293 cells and were purified by double cesium chloride gradient. AAV vectors were administered into the pancreatic duct through the sphincter of Oddi while the choledoc was clamped close to the liver edge.
Results: The intraductal injection of several doses of AAV6, AAV8 and AAV9 vectors coding GFP resulted in an efficient transduction of the exocrine and endocrine pancreas. The number of transduced acinar cells increased in a dose dependent manner and AAV9 was the most efficient serotype transducing these cells. Transduction of ductal cells was only observed when AAV6 was administered intraductally. AAV6, AAV8 and AAV9 vectors administered via the pancreatic duct transduced beta cells in the periphery of the islets. Moreover, beta cells in the periphery and in the core of the islets showed high expression of the marker gene when high doses of AAV6, AAV8 and AAV9 were administered to the pancreas. However low percentage of alpha cells were transduced by AAV serotypes 6, 8 and 9. Furthermore, the intraductal administration of AAV9 vectors coding mHGF resulted in partial prevention of T1D in a transgenic animal model of diabetes.
Conclusion: These results show that AAV administration via the pancreatic duct is an efficient approach for genetic manipulation of the pancreas. This approach could be used to study islet and pancreas physiology and also to assay new gene therapy approaches for Diabetes Mellitus and other pancreatic disorders.
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Administration of an IL-1 trap counteracts type 1 diabetes in NOD mice

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Background and aims: We previously reported that the addition of IL-1 traps (hybrid molecules consisting of the extracellular domain of IL-1 receptor accessory protein and IL-1 receptor type 1 arranged inline and fused to the Fc-portion of IgG1) to rat pancreatic islets *in vitro* can protect against noxious effects induced by IL-1β as well as a cytokine combination. In this study we tested the effect of administration of a murine IL-1 trap on the recurrence of disease (ROD) model in non obese diabetic (NOD) mice.
Materials and methods: Spontaneously diabetic female NOD mice received implantation of a curative number (600) of syngeneic pancreatic islets, from young healthy NOD mouse donors, beneath their left kidney capsule. Once a day, the mice were injected subcutaneously with IL-1 trap (30 mg/kg bodyweight), or an equimolar dose Fc-control protein (8.4 mg/kg bodyweight) or saline. The treatments were maintained until ROD (i.e. a blood glucose value >11.1 mM for 2 consecutive days) or until 5 days after transplantation. Data are presented as means ± SEM.
Results: Analysis of cumulative islet graft survival revealed that mice treated with IL-1 trap had an increased graft survival (14.1 ± 3.2 days) compared to mice treated with Fc-control protein (5.6 ± 1.0 days) ($p<0.05$), when compared with the Kaplan-Meier survival curve and Logrank test, and a trend for prolonged graft survival compared to saline treated mice (7.6 ± 0.9 days) ($p=0.076$). Analysis of relative cytokine mRNA levels in isolated spleen cells, retrieved after both endpoints, showed elevated IL-4 mRNA levels in cells from mice treated with IL-1 trap, compared to both control groups (endpoint ROD: Saline 0.0072 ± 0.0008, Fc-protein 0.0101 ± 0.0019, IL-1 trap 0.0239 ± 0.0063*, endpoint 5 days post tx: Saline 0.0059 ± 0.0009, Fc-protein 0.0042 ± 0.0004, IL-1 trap 0.0142 ± 0.0041* ($2^{-\Delta CT}$), n=5-7, *=$p<0.05$ vs. both Saline and Fc-protein treated animals using one way ANOVA with subsequent all pair-wise comparison procedures by Student-Newman-Keuls method). This suggests a shift toward Th2 cytokine production in the IL-1 trap treated animals, which may contribute to its protective effect.
Conclusion: Administration of an IL-1 trap counteracts islet cell destruction in this recurrence of disease NOD mouse model of type 1 diabetes.
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Ciliary neurotrophic factor prevents, delays and ameliorates type 1 diabetes in Swiss, C57Bl6 and iNOS-/- mice

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Background and aims: CNTF is a cytokine known for its survival effects in many cell types, including rat pancreatic islets. Type 1 Diabetes (DM1) is characterized by a selective loss of pancreatic islet beta-cell mass and subsequent hyperglycaemia, a condition that can be induced by Multiple Low Doses of Streptozotocin (STZ), a model (MLDS) that resembles human Type 1 Diabetes. The Nitric Oxide (NO) production and Inducible Nitric Oxide Sintase (INOS) expression role on the DM1 onset and progression have been controversial. Given CNTF beta-cell protection against apoptosis, we decided to evaluate the effects of CNTF in a Type 1 Diabetes model that resemble the human disease (MLDS), and since CNTF was described to increase INOS expression and NO production, we assessed whether or not its effects on DM1 would be hampered in a INOS Knockout mice.
Materials and methods: For MLDS, 4-6 weeks-old Swiss, C57Bl6 or INOS-/- mice were administered by intra-peritoneal injection once daily for 5 consecutive days using saline injection as control (C), 40mg/Kg of STZ for MLDS group (S) 40mg/Kg of STZ plus 0.1 mg/Kg of CNTF for CNTF group (N). We assessed mice fed glycaemia in days -3, 0, 1, 3, 7, 14, 21, 28 and 35 after the first injection. Type 1 diabetes considered as blood glycaemia >250 mg/dl for 2 days. We evaluated mice overall glycaemia, Type 1 diabetes incidence, severity and time until occurrence. Furthermore, we evaluate INOS mRNA expression in MIN6 cells cultured for 3 days in the absence (CTL) or presence 0.1nM, 1nM or 5nM of CNTF by RT-PCR (n=16). Data=Mean +-SEM. $P<0.05$.
Results: In all strains (Swiss, C57Bl6 and iNOS-/-), CNTF-treated mice had an overall lower glycaemia than STZ-group and a lower Type 1 diabetes incidence, although it was more pronounced in the C57Bl6 one. Even among those that developed diabetes in CNTF group, it took them longer to occur and was less severe than the STZ group. CNTF increased INOS expression in MIN6 cells in a dose-dependent manner, but even so, its protective effects against DM1 in INOS-/- strain were similar to those observed in the wild-type C57BL6.
Conclusion: The results indicate that CNTF protects against STZ-induced Type 1 Diabetes, even in INOS-/- mice strain. Given that CNTF increased MIN6 cells INOS expression in a dose-dependent manner, the CNTF protective effects were not mediated trough or hampered by the increased INOS expression, and the functional importance of this increase in INOS expression by CNTF in mice beta-cells is yet to explained. Lastly, we can conclude CNTF could be a potential therapeutic tool in the prevention or even early treatment of DM1 patients.
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Induction of regulatory CD8 T cells after diabetes manifestation by treatment with a rat specific CD3 antibody in the IDDM rat as a tool for protection of pancreatic beta cell function

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Background and aims: In human type 1 diabetic patients the residual function of the beta cells, measured by C-peptide, can be restored by treatment with CD3-antibody (AB) for up to one year. Interestingly, in humans it was found that the number of regulatory CD8 T-cells in the blood was increased after therapy with CD3-AB. In this study we treated IDDM (LEW.1AR1-iddm) rats, an animal model of type 1 diabetes mellitus (T1DM), immediately after diabetes manifestation with CD3-AB in analogy to the human therapy. The aim of the study was to analyse changes of the immune cell subpopulations in the blood and pancreas draining lymph nodes of animals treated with CD3-AB in comparison to acutely diabetic animals without therapy and normoglycaemic rats.
Materials and methods: To secure beta cell function after diabetes manifestation, acutely diabetic IDDM rats were treated with CD3-AB (Clon R73 0.5 mg/kg b. wt.) consecutively over 5 days. The effect of the CD3-AB therapy and changes in the immune cell subpopulations were analysed by flow cytometric analysis in the blood and by real time PCR analysis of isolated T-cells from pancreas draining lymph nodes. The infiltration stages of the pancreatic islets in CD3-AB treated IDDM rats were compared to normoglycaemic IDDM rats and diabetic rats without treatment.
Results: In prevention therapy with CD3-AB, initiated directly after diabetes manifestation, the reduction of all T-cells can be monitored by flow cytometric analysis. FoxP3 positive T-cells, especially CD8 T-cells, increased within one week of therapy. The increased number of regulatory CD8 T-cells induced by CD3-AB therapy was observed in all treated IDDM rats in comparison to acutely diabetic rats even though the efficacy of the prevention therapy with CD3-AB was dependent of the metabolic state of the diabetic IDDM rats before start of treatment. Only animals with a blood glucose concentration up to 13 mmol/l at the start of the therapy could be completely cured. After therapy the number of pancreatic beta cells increased in the islets of these animals whereas the signs of immune cell infiltration were less reduced in comparison to the diabetic animals without treatment. Even after a complete clinical remission of diabetes, a certain immune cell infiltration remained in the islets.
Conclusion: Interestingly, analogues to the findings in type 1 diabetic patients under CD3-AB therapy, the IDDM rats showed also an increased number of regulatory CD8 T-cells in the blood. This indicates that the IDDM rat is a very good model to analyse the function of regulatory T-cells in the context of CD3-AB treatment to modulate the imbalance of the immune cells during diabetes development by an increase of regulatory T-cells.

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Comparison of two type 1 diabetes prevention therapies of a CD3 antibody in combination with FTY720 or a TNF-α antibody on beta cell function in the IDDM rat, an animal model of type 1 diabetes

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Background and aims: The IDDM (LEW.1AR1-*iddm*) rat is an animal model of type 1 diabetes mellitus without lymphopenia and therefore very suitable for evaluation of combined immunomodulatory therapies. The aim of the CD3-antibody (AB) treatment in combination with the immunomodulatory agent FTY720 or the TNF-α-AB was to protect the remaining pancreatic beta cells from autoimmune destruction after onset of the disease. The CD3-AB interacts with the T-cell receptor to inhibit cytokine production and release from T-lymphocytes, while the sphingosin-1-phosphate receptor antagonist FTY720 retains the lymphocytes in the organ draining lymph nodes and the TNF-α-AB blocks the released TNF-α.
Materials and methods: Animals were treated with CD3-AB (0.5 mg/kg body weight) in combination with either TNF-α-AB (1 mg/kg b. wt.) consecutively over 5 days or over 40 days with FTY720 (1 mg/kg b. wt.). Besides the metabolic changes, pancreatic biopsies at the time point of diabetes manifestation, at the end of therapy, and 60 days after the end of therapy were analysed for beta cell survival and immune cell infiltration by ultrastructure as well as on the gene and protein expression level.
Results: Prevention therapy with both combinations starting immediately after disease manifestation with blood glucose concentration values between 10 - 15 mmol/l reversed clinical diabetes leading to normoglycaemia during and after therapy. Morphologically, beta cells in the islets during and after therapy showed a fivefold reduced apoptosis rate and a threefold increased islet beta cell area. Both CD8 T-cells and CD68 macrophages were markedly decreased in the islets at the end of therapy and nearly completely lost 60 days later. The small amount of immune cells surrounding the islets and in the capillary system of the islets lost the pro-inflammatory cytokine expression after therapy. In both combined therapies with the immunomodulatory agents the remaining beta cells in the islets were ultrastructurally well preserved without signs of destruction. The only difference between both combination therapies was the higher amount of macrophages in and around the islets after CD3- and TNF-α-ABs-therapy.
Conclusion: Combined therapy of CD3-AB with FTY720 or with TNF-α-AB showed a great benefit with respect to the survival of pancreatic beta cells with a normalized apoptosis rate in islets without immune cell infiltration in comparison to the initial small number of beta cells and the high apoptosis rate in the severely infiltrated islets before the start of the therapy. The beneficial effects of both strategies could be maintained also after the end of the therapy.

PS 21 Islet autoantibodies in type 1 diabetes

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Combined measurement of autoantibodies against GAD, IA-2, insulin and ZnT8 in the Diabetes Autoantibody Standardization Program 2009 Workshop

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Background and aims: The Diabetes Autoantibody Standardization Program (DASP) is a collaboration between the Immunology of Diabetes Society and US Centers for Disease Control and Prevention, set up to evaluate and improve assays for type 1 diabetes (T1D)-associated autoantibodies (AAb). The DASP 2009 workshop aimed to assess the sensitivity/specificity and concordance of assays measuring AAb to GAD (GADA), IA-2 (IA-2A), insulin (IAA), and zinc transporter 8 (ZnT8A) in laboratories throughout the world. We assessed the combined detection of these four AAb in DASP serum samples considering differences in assay performance.
Materials and methods: Coded sera from 50 patients with newly diagnosed T1D and 100 healthy controls were analyzed in laboratories from 19 countries by 53 GADA assays, 52 IA-2A assays, 31 IAA assays and 19 ZnT8A assays. Six control sera that were found positive for multiple AAb or with very high titre GADA in >90% of laboratories were excluded from this analysis. Sera were categorized according to the number of laboratories calling them positive using local assay thresholds, and further stratified between patients and controls by the number of positive AAb. Category 1 (C1) included sera that were found AAb positive by at least 90% of assays. Category 2 (C2) included C1-sera plus additional sera that were found AAb positive by at least 50% of assays. Category 3 (C3) included C2-sera plus sera that were found AAb positive by at least 25% of assays.
Results: Of 50 patient sera, 41(82%) were included in C1, 45 (90%) in C2 and 46 (92%) in C3. None of the 94 control sera were included in C1 (0%), while 7 (7%) and 13 (14%) control sera were included in C2 and C3 respectively. The number of multiple AAb positive patient sera increased from 24 (59%) in C1, to 34 (76%) in C2, and 41 (89%) in C3 (p=0.001). One control serum in C3 was positive for multiple AAb. For all AAb specificities, C1-sera had significantly higher antibody titres than C2-sera (p<0.0001). Among the 46 AAb positive patient sera in C3, 18 (39%) had all four AAb, and another 15 (33%) had three positive AAb, most frequently as the combination of GADA plus IA-2A plus ZnT8A. Among single AAb positive sera, GADA were most frequently detected in both, patients (n=4) and controls (n=6).
Conclusion: We conclude that laboratories with sensitive assays for GADA, IA-2A, IAA, and ZnT8A could detect AAb in almost all sera from newly diagnosed T1D patients in the DASP 2009 workshop, and multiple AAb in up to 90% of AAb positive patient sera. AAb assays may infrequently detect positive signals against single but usually not multiple antigens in sera from controls. Discrepancies in autoantibody measurement were found to occur most commonly for low titre GADA samples and identify a subset worthy of further study.

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Identification of autoantibody to Zinc Transporter 8 in Japanese diabetic patients

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Background and aims: Zinc transporter 8 (ZnT8) is expressed specifically on the membrane of insulin secretary granules in pancreatic β-cells. Recently, autoantibody to ZnT8 (ZnT8Ab) was reported to be implicated in type 1 diabetes especially in Caucasian. This study aimed to evaluate the clinical significance of ZnT8Ab in Japanese patients with diabetes.
Materials and methods: Sera from 1316 diabetic patients (258 from type 1 diabetes (T1D), 848 from type 2 diabetes (T2D), 210 from latent autoimmune diabetes in adults (LADA)) were collected in Saitama Social Insurance Hospital, and control sera were collected from 177 healthy subjects without any histories of diabetes or autoimmune diseases. ZnT8Ab titer was measured by radioimmunoprecipitation assay using a recombinant ZnT8 C-terminal peptide (aa268-369, Arg325; CR) or a chimeric construct of CR and CW (Trp325) (CR-CW) as antigens (kindly provided by Prof. Hutton, Colorado Univ.).
Results: Sera from healthy subjects were 1.1% positive to CR and 1.7% to CR-CW. The positive rate of ZnT8Ab was 2%, 19% and 38% in T2D, LADA and T1D, respectively. In case of T1D, the positive rate decreased markedly after 2 years from the onset; therefore, we selected and analyzed samples within one year from the onset. In this instance, the positive rate of T1D was 51% (n=94). Moreover, the positive rate was higher in age group from 13 to 19 years old (82%) than that over 20 years old (44%) (p = 0.004). We then examined the association between ZnT8Ab and other autoantibodies (GADAb, IA-2Ab) in T1D. Among sera positive to ZnT8Ab, GAD antibody (GADAb) and IA-2 antibody (IA-2Ab) was detected in 88% and 68%, respectively. Alternatively, sera negative to ZnT8Ab exhibited 67% and 36% positive to GADAb and IA-2Ab, respectively. In T1D, the prevalence of GADAb as a single autoantibody was 77%, and double positive rate with IA-2Ab was 80%. Further addition of ZnT8Ab increased the rate up to 85%. In clinical respects, serum C-peptide demonstrated a significant difference between ZnT8 positive patients and negative patients (0.75±0.57 vs. 0.96±0.66; p=0.024). And body mass index of ZnT8Ab positive patients was lower than that of negative patients (19±2.1 vs. 21±3.4; p=0.005).
Conclusion: In Japanese patients with diabetes, ZnT8Ab was positive in 51% of T1D and 2% of T2D. In T1D, patients in the age from 13 to 19 demonstrated higher positive rate than elder groups. ZnT8Ab seemed to be associated with other autoantibodies, especially such as IA-2Ab, suggesting its similar feature. Combination of ZnT8Ab in addition to GADAb and IA-2Ab increased the positive rate of autoantibodies in T1D by 5%. We supposed that ZnT8Ab might become a useful marker in Japanese diabetic patients.

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The catalytic cysteine of islet antigen 2 is crucial to autoantibody binding in type 1 diabetes

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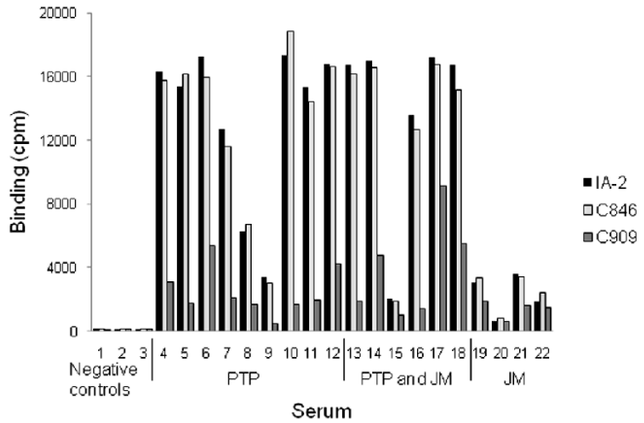
Background and aims: Autoantibodies to islet antigen 2 (IA-2A) are important for characterising the prodrome of type 1 diabetes (T1D) and underpin current methods of disease prediction. IA-2A show selective binding to dominant epitopes in the juxtamemebrane (JM) and protein tyrosine phosphatase (PTP) regions of IA-2. We have shown that azide and high concentrations of Tween-20 cause large reductions in binding to PTP epitopes of IA-2, possibly by modification of cysteine residues. This could disrupt disulphide bonds or modify the catalytic cysteine of the PTP active site. Our aim was to identify which cysteine residues are critical to autoantibody binding in patients with T1D.
Materials and methods: Candidate cysteine residues within a major epitope region (position 846) and the catalytic cysteine (position 909) of IA-2ic (606-979) were substituted with serine by site-directed mutagenesis. The effects of these changes on autoantibody binding were investigated by radiobinding assay using in vitro transcribed and translated 35-S labelled antigens (20,000cpm) with; (1) A panel of 22 well-characterised samples which included 19 IA-2A positive sera from 15 patients with T1D, 3 T1D relatives and 1 T1D standard pool, as well as IA-2 negative sera from 3 healthy controls (2) sera from 35 IA-2A positive patients (19 male) with newly diagnosed T1D (median age 11, range 2 to 19 years).
Results: (1) Mutation of the catalytic cysteine at position 909 caused a large reduction in IA-2A binding by the 19 well-characterised IA-2A positive sera (Figure); median 73%, range 7 to 90%, (p<0.001, Wilcoxon signed rank test), affecting sera positive for antibodies to both PTP and JM regions. In contrast, cysteine substitution at position 846 had no effect; median 3%, range -31 to 12% (p=0.167). (2) In IA-2A positive patients with T1D, mutation of cysteine 909 caused a median reduction in binding of 60%, range 21 to 79%,

(p<0.001). Mutation of cysteine at 846 again had no significant effect; median 3%, range -4 to 16% (p=0.091).
Conclusion: Mutation of the catalytic cysteine causes profound reduction in IA-2A binding by sera from patients with T1D and identifies a novel site that is critical to epitope integrity in the cytoplasmic region of IA-2. These findings indicate that the catalytic cysteine is a crucial residue in determining the conformation of IA-2.



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Characterisation of IA-2 antibody epitopes by proteolysis of antibody-antigen complexes and mass spectrometry
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Background and aims: Destruction of pancreatic beta cells in Type 1 diabetes is T-cell-mediated but circulating autoantibodies are also strongly associated with disease progression. Autoantibody-secreting B-cells participate in diabetes pathogenesis by facilitating antigen processing and presentation and are potential targets for diabetes therapy. Knowledge of autoantibody epitopes will aid the development of antigen-specific, B-cell targeted immune intervention. We have two monoclonal autoantibodies from diabetic patients that recognise dominant epitopes on IA-2, a major target of autoimmunity in the disease. Limited information on these epitopes has been obtained by amino acid substitution experiments. The aim of this study was to further define the antibody binding region by mass spectrometric identification of peptides remaining bound to antibody after proteolysis of antibody-antigen complexes.
Materials and methods: Recombinant protein representing the tyrosine phosphatase (PTP) domain of IA-2, either free or complexed with the Sepharose-bound monoclonal antibodies, were digested with chymotrypsin and antibody-bound peptides eluted at pH 11.7. Peptides were analysed by matrix assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry and identified by mass comparison against a database of IA-2 chymotryptic products. Peptide identity was confirmed by liquid chromatography-tandem mass spectrometry. Acceptance criteria included a mass tolerance of less than 1.5 Da and X-correlation score of greater than 2 for doubly charged ions and greater than 2.5 for triply charged ions.
Results: Mass spectrometry of chymotryptic digestion products of free IA-2 PTP domain identified peptides with broad coverage (78%) of the molecule, whereas peptides eluted from the two monoclonal antibodies were restricted to four regions spanning positions 765-795, 813-832, 833-854 and 855-873. The latter 3 regions are adjacent on the surface of a model of the IA-2 PTP domain and encompass residues previously shown to be important in antibody binding using site directed mutagenesis. The 765-795 region is largely buried within the protein and lies directly beneath the regions previously mentioned. Six cleavage sites (at positions 794, 823, 839, 850, 856 and 872) detected with free IA-2 were never cleaved in peptides eluted from the antibodies, suggesting that antibody protects these sites from protease digestion.
Conclusion: Using antibody footprinting followed by mass spectrometry, we have mapped regions on IA-2 that represent major epitopes for diabetes-associated autoantibodies in Type 1 diabetes, confirming and extending previous studies using site-directed mutagenesis. Peptides remaining antibody-bound after proteolytic digestion closely overlap with areas of known T-cell reactivity in Type 1 diabetes, supporting the concept that B-cells influence the T-cell response through modulation of antigen processing.
Supported by: JDRF

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Formation of insoluble immune complexes impairs detection of type 1 diabetes-associated autoantibodies in radioligand binding assays
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Background and aims: Detection of autoantibodies associated with Type 1 diabetes is commonly achieved using radioligand binding assays. In these assays, complexes of serum autoantibodies with radiolabelled autoantigens are captured on protein A-Sepharose prior to quantification by scintillation counting. We have observed that a proportion of Type 1 diabetic patients show up to 5-fold higher recoveries of immunoprecipitated IA-2 or glutamic acid decarboxylase when serum antibodies are pre-bound to protein A Sepharose prior to incubation with antigen (solid phase assay) than when immune complexes are formed in solution and subsequently captured on protein A Sepharose (liquid phase assay). These observations suggested either the presence of a serum factor inhibiting autoantibody-antigen interactions, or poor binding to protein A Sepharose of immune complexes with autoantibodies of some diabetic patients. The aim of this study was to investigate the cause of these differences using IA-2 as the target antigen.
Materials and methods: Sera were selected from Type 1 diabetic patients showing higher antibody reactivity to IA-2 in the solid phase compared to liquid phase assay. IgG fractions were isolated from sera by protein A-Sepharose affinity chromatography and IA-2-specific autoantibodies affinity purified on IA-2-conjugated Sepharose and eluted at pH 11.7. Fab fragments of IgG were generated by papain cleavage. Antibody binding to radiolabelled IA-2 was analysed in two assay formats: i) where antibodies were pre-bound to protein A Sepharose and washed before addition of in vitro transcribed and translated IA-2 (40,000 cpm per reaction) for 4 h or ii) where antibodies were incubated for 4h in liquid phase with the radiolabelled IA-2 before protein A Sepharose addition. Protein A Sepharose-bound complexes were washed by centrifugation (5,000 cpm for 30 secs on microfuge) before scintillation counting. Insoluble immune complexes were isolated by centrifugation at 13,000 rpm for 20 mins and washed with 5% polyethylene glycol. All incubations were performed at 4C.
Results: Increased IA-2 antibody binding in solid phase assay was observed with both serum and protein A sepharose-purified antibodies from diabetic patients, indicating that any factor that may inhibit antibody binding was present in IgG fraction. Inhibitory effects of anti-idiotypic antibodies were shown to be unlikely because Fab fragments of IA-2 antibody-depleted IgG failed to block binding of affinity-purified antibodies to IA-2 in liquid phase assays. However, we were able to demonstrate the presence of radiolabelled IA-2 within insoluble immune complexes isolated from supernatants from the liquid phase assays. These insoluble immune complexes failed to bind protein A Sepharose. Radiolabelled IA-2 was not detected in insoluble immune complexes isolated from solid phase assays.
Conclusion: This study demonstrates that a proportion of Type 1 diabetic patients possess circulating IA-2 autoantibodies that form insoluble immune complexes with antigen with poor binding to protein A. Assays dependent on protein A capture of immune complexes may underestimate autoantibody levels in those patients and these observations need to be considered when designing new protocols for autoantibody detection. The ability to form insoluble immune complexes in vivo may have implications for tissue damage and the regulation of autoimmune responses in the diabetic patient.
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Seroconversion to persistent antibody-positivity occurs frequently after age 10 and is best predicted by GAD antibodies
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Background and aims: The appearance of autoantibodies (Ab^+) before clinical diabetes onset has mainly been studied in children. However, most patients develop type 1 diabetes in adulthood. We studied seroconversion to Ab^+ in first-degree relatives aged 0-39 years to determine 1) age distribution

at seroconversion; 2) predictors of Ab-persistence and prediabetes; 3) order of appearance of different Ab-types.
Materials and methods: Seroconverters (n=221) were identified by measuring Abs against insulin (IAA), islet cell cytoplasm (ICA), GAD (GADA), IA-2 (IA-2A) and zinc transporter 8 (ZnT8A-CRCW) during follow-up of 6125 first-degree relatives.
Results: One hundred and five (48%) relatives seroconverted to persistent Ab^+. Most seroconversions (63%) occurred after age 10. Persistently Ab^+ relatives were more frequently $GADA^+$ (61% vs 23%) and multiple Ab^+ (26% vs 2%) (resp. $p<0.001$ and $p<0.008$ by logistic regression) at seroconversion than transiently Ab^+ relatives. The prevalence of IA-2A and ZnT8A was initially the lowest (resp. 12% and 7%) but increased most thereafter (resp. +100% and +214% vs +24% for IAA and +11% for GADA). Progression to diabetes occurred almost exclusively (20/22) in persistently Ab^+ relatives ($p<0.001$ by logrank vs transiently Ab^+) and preferentially in case of seroconversion under age 10 ($p<0.021$). Still, 9 (41%) prediabetics, had seroconverted after age 10.
Conclusion: 1) Seroconversion after age 10 is not a rare event; 2) GADA is the best predictor of Ab-persistence which is in turn associated with progression to diabetes; 3) in case of Ab-persistence, IA-2A and ZnT8A preferentially mark later disease stages, compatible with their known association with rapid progression to diabetes.
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Frequency of development of type 1 diabetes mellitus and immunity indices in prediabetic children of Ukraine who are positive to autoantibodies to Langerhans islet antigens
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Background and aims: The discovery of human diabetes-associated autoantibodies (DAAb) contributed to an early prediction of development of type 1 diabetes mellitus (T1DM) and to investigations of the immunological mechanisms of its progression to a latent stage. In 1998, for the first time in Ukraine a Programme "Immunity at preclinical stage of T1DM progression" has been developed by the Institute, whose purpose was a prospective study of the frequency of T1DM development and dynamics of changes in several immunity indices in prediabetic period among children of a number of regions of Ukraine, who have a family history of diabetes and are positive or negative to the presence of DAAb.
Materials and methods: 450 children aged 13.62±2.34 years were followed up. Out of 366 practically healthy normoglycemic children with first degree relatives having T1DM, 94 were positive (group A) and 272 (group B) negative to DAAb (GADA and IA-2A). A control group C comprised 84 normoglycemic children without a family history of diabetes and being DAAb-negative. The measurements of IAA, GADA, and IA-2A titers were carried out by radioimmunoassay, the immunophenotype of lymphocytes (CD3+, CD4+, CD8+, CD20+, and CD56+ cells) by FACS-analysis, the content of cytokines (IL-1, IL-4, IL-6, IL-8, IL-10, IL-16, TNFα, and IFN-γ) by ELISA.
Results: T1DM has appeared in different periods of follow-up (from 6 months to 9 years: 30.9±3.2 months) in 48.9% of children from group A and in one child (0.8%) from group B. Among relatives of group A subjects, in 55.3% of children the sibs, in 36.1% the father, and in 9.6% the mother, had diabetes. Group A, unlike groups B and C, was characterized by a lower absolute number of content of CD3+ ($0.79\pm0.05 \times 10^9$/L), CD4+ ($0.52\pm0.03 \times 10^9$/L), and CD56+ ($0.18\pm0.02 \times 10^9$/L) cells in peripheral blood compared to group C ($1.39\pm0.09 \times 10^9$/L; $p<0.01$; $0.98\pm0.04 \times 10^9$/L; $p<0.001$; $0.29\pm0.02 \times 10^9$/L; $p<0.01$, respectively), and group B ($1.02\pm0.06 \times 10^9$/L; $p<0.05$; $0.67\pm0.04 \times 10^9$/L; $0.24\pm0.03 \times 10^9$/L; $p<0.01$, respectively), and by a higher concentration of circulating IL-1α, IL-6, IL-8, IL-16, TNFα and a decreased IL-4, with a higher degree of changes of these indices at preclinical stage in a number of patients than after disease manifestation. In children from group A, whose father had T1DM and in which a higher simultaneous increase in GADA and IA-2A titers correlated with the most pronounced decrease in the content of IL-4 and CD3+ cells ($0.59\pm0.05 \times 10^9$/L; $p<0.01$) and of CD4+ cells ($0.38\pm0.03 \times 10^9$/L; $p<0.01$), CD56+ cells ($0.15\pm0.03 \times 10^9$/L; $p<0.01$), and with elevated levels of IL-16, IL-8, TNFα and IFN-γ, a faster progression of T1DM (16.32±4.45 months vs 30.9±3.2 months, $p<0.01$) was reported, along with a more aggressive character of disease course.
Conclusion: The frequency of development of T1DM in DAAb+ normoglycemic children of Ukraine is near the upper limits of this index for other countries. The velocity of T1DM progression and aggressiveness of disease course depend not only on the presence of a higher DAAb titer and the degree of changes in T- and NK-immunity indices, but on the presence of a history of diabetes in one of the parents as well.

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Role of diabetic family history in the prediabetic autoimmune process in children recruited from the general population based on HLA-associated disease risk
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Background and aims: To assess differences in the in the appearance and progression of beta-cell autoimmunity between children with and without first-degree relatives (FDRs) affected by type 1 diabetes (T1D) in a study cohort recruited from the general population based on HLA-conferred disease risk.
Materials and methods: 7410 children with HLA-conferred disease risk were recruited at birth from the general population and observed for 9.2 years (median) for beta-cell autoimmunity and T1D. At birth 177 (2.4%) of the children had affected FDRs. ICA were used for autoantibody screening. After ICA-positivity or T1D was confirmed, all samples available from that individual were analyzed for ICA, IAA, GADA, and IA-2A. Children with advanced autoimmunity (persistent positivity for ≥2 diabetes-associated autoantibodies, DAAs) were eligible for an intervention trial with nasally administrated insulin/placebo that proved to have no effect on progression to T1D.
Results: Children with affected FDRs seroconverted to autoantibody positivity and developed persistent multipositivity more frequently, and seroconverted at a younger age than children without FDRs with T1D (Table 1). They had also higher autoantibody levels throughout the follow-up. However, in children with advanced multipositivity autoantibody levels were similar regardless of family history. In children with affected FDRs, paternal T1D was associated with higher risk of seroconversion (paternal vs. maternal/sib: OR 2.0 [$CI_{95\%}$ 1.0-3.9], P=0.04), persistent multipositivity (OR 2.2 [$CI_{95\%}$ 1.0-5.0], P=0.048), and T1D (OR 4.5 [$CI_{95\%}$ 1.7-11.7], P=0.002) than maternal T1D or T1D in siblings. For comparison, OR for T1D in children with paternal T1D vs. children without affected FDRs was 13.4 ($CI_{95\%}$ 7.8-23.1).
Conclusion: The most conspicuous effect of familial T1D is seen in the initiation of the preclinical disease process. Children with paternal T1D are at the highest risk of developing beta-cell autoimmunity and T1D. After the diabetic autoimmune process is established, the FDR status plays a minor role in terms of disease progression.

Table 1. Beta-cell autoimmunity in children with HLA-conferred disease risk in relation to family history of type 1 diabetes (T1D).

	Family history of T1D	
	Positive	Negative
	N (%)	
ICA-based seroconversion	49 (27.7)	1124 (15.5)*
Seroconversion sample multipositive	16 (32.7)	145 (12.9)*
Positivity for ≥2 DAAs	38 (21.5)	353 (4.9)*
Persistent autoantibody positivity	40 (22.6)	632 (8.7)*
Persistently positive for ≥2 DAAs	31 (17.5)	252 (3.5)*
Progression to T1D	24 (13.6)	156 (2.2)*
	Years, median (range)	
Age at seroconversion	3.0 (0.3–10.5)	4.0 (0.2–13.7)†
Delay from seroconversion to diagnosis of T1D	2.8 (0.3–8.6)	2.8 (0.0–10.9)
Age at diagnosis (all children with T1D)	4.8 (0.9–11.4)	5.1 (1.0–12.5)

*P≤0.001, †P=0.02.

*P≤0.001, †P=0.02. ICA, islet cell antibodies; IAA, GADA, and IA-2A: autoantibodies against insulin, glutamate decarboxylase, and islet antigen 2, respectively.

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Effect of family history of type 1 diabetes on the phenotype of type 2 diabetes

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Background and aims: Type 2 diabetes (T2D) is a heterogeneous disease and 10- 20 % of patients develop marked insulin deficiency. Some have GAD antibodies (LADA, latent autoimmune diabetes in adults) and share susceptibility genotypes with type 1 diabetes. In Finland, Type 1 and Type 2 diabetes cluster in same families. We evaluated the impact of family history of diabetes and susceptibility genotypes on the phenotype of type 2 diabetic patients.
Materials and methods: We recruited 197 T2D patients with family history of T1D and 139 matched (age, age of onset, BMI, sex) T2D controls with family history of T2D only. Phenotypical data included an oral glucose tolerance test (OGTT) and i.v. glucagon test followed by i.v. insulin tolerance test (GITT) and GAD antibodies (GADA). Patients were genotyped for T1D (*HLA-DQA1-DQB1, PTPN22, INS*) and T2D (*TCF7L2,FTO, PPARG, KCNQ1, SLC30A8*) susceptibility genes. Patients were stratified according to T1D family history and GADA positivity.
Results: There were altogether 58 GADA+ T2D patients; 43 with T1D family history ($LADA_{MIX}$; 30 had 1st degree relatives with T1D) and 17 with family history for T2D only ($LADA_{T2}$), p=0.024. The $LADA_{MIX}$ patients had higher HbA1c and glucose values during OGTT than both $LADA_{T2}$ and GADA- T2D patients with mixed family history ($T2D_{MIX}$) (table 1). They were less obese and they had lower measures of insulin secretion (fasting and glucagon-stimulated C-peptide, corrected insulin response during 30 min of OGTT, CIR) even when adjusted for the degree of insulin resistance (Disposition index). 37.2% of the $LADA_{MIX}$ patients had markedly decreased stimulated C-peptide (< 0.7 nmol/l) compared to 10.4% of $T2D_{MIX}$ and 4.9% of $T2D_{T2}$ patients(p<0.001). Surprisingly the $LADA_{MIX}$ patients also seemed to be more insulin resistant (higher HOMA and lower K_{ITT} during the insulin tolerance test), but this could be due to worse diabetes control. $LADA_{MIX}$ patients had significantly more often *HLA-DQ* T1D risk genotypes 19/43 (44.2%) compared with $T2D_{MIX}$ 39/154 (25.3%; p=0.05) and $T2D_{T2}$ 17/121 (14.0%; p=0.0003).
Conclusion: TID family history together with GADA positivity is associated with insulin deficiency in type 2 diabetic patients.

Table: Data are means ± SD or median (IQR). In statistical analyses (Ancova) the data were adjusted for sex. *p<0.05, **p<0.001 $LADA_{MIX}$ vs $T2D_{MIX}$, † p<0.05, †† p<0.001 $LADA_{MIX}$ vs $LADA_{T2}$

Clinical characteristics

	$LADA_{T2}$ (n=17)	$LADA_{MIX}$ (n=43)	$T2D_{MIX}$ (n=156)	$T2D_{T2}$ (n=122)
BMI (kg/m)	29.3±2.3	28.2±4.2	29.8±5.7	30.4±4.8
GADA (IU/ml)	42[58] ††	†† 89.0[2136]**	12.0[9.25]**	16.0[12.0]
Diabetes duration (years)	6.0[9.5]	7.0[11.0]	6.0[8.3]	8.0[8.0]
Diabetes onset (years)	50.0[16.0]	49.0[13.0]	52.0[12.3]	52.0[10.3]
HbA1c (%)	6.9 ± 1.) †	† 7.7 ± 1.5**	6.9 ± 1.1**	6.8 ± 1.0
FPG (mmol/l)	8.2 ± 2.7	8.4 ± 2.6	7.9 ± 2.0	7.5 ± 1.8
$C\text{-peptide}_{GITT}$ 6 min (nmol/l)	1.5[0.8] †	† 1.0[1.4]**	1.4[0.9]**	1.4[0.8]
$HOMA_{OGTT}$	2.3[4.2]	2.5[2.0]*	3.3[3.2]*	3.0[2.8]
CIR	21.9[20.0]	18.0[24.1]*	22.7[22.2]*	22.0[23.5]
$Disposition\ index_{ITT}$	2.7[1.8]††	†† 1.6[2.2]**	2.6{2.3]**	2.7[2.3]
K_{ITT}	2.0[1.1]	1.5[1.4]*	1.9[1.3]*	2.0[1.3]

PS 22 T regulatory cells and Th17 immunity in type 1 diabetes

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IL-17 immunity in human type 1 diabetes

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Background and aims: T1D is considered as an autoimmune disease caused by T-cell mediated destruction of the insulin-producing pancreatic beta-cells. Th17 immunity has been demonstrated in the development of autoimmune diabetes in animal models. IL-17 neutralization prevented development of diabetes in NOD mice. Moreover, islet-cell antigen specific Th17 cells converted into IFN-γ secreting Th1-like cells and caused diabetes in mice recipients. Our aim was to address the role of Th17 cells in human T1D.
Materials and methods: We studied 15 children with T1D and 12 non-diabetic children for the comparison of IL-17 immunity in PBMCs stimulated with plate-bound anti-CD3 and soluble anti-CD28 for 40h. Culture supernatants were collected for cytokine analysis and stimulated cells were collected for gene expression analysis of IL-17, ROR-C2, IL-22, IFN-γ, T-bet and FOXP3 with qRT-PCR. Th17 immunity associated genes in freshly purified CD4+ memory T-cells were also compared between nine T1D patients and eight healthy children. CCR6, TCR-αβ, TCR-γδ and intracellular IFN-γ expression of IL-17 producing cells were analysed by flow cytometry. The apoptotic and inflammatory effects of IL-17 alone or in combination with IL-1β and IFN-γ on human islets from five different donors were studied in vitro with qRT-PCR. The proportion of apoptotic islets cells were determined by nuclear double staining with Hoechst 33342 and propidium iodide.
Results: We observed increased IL-17 secretion (p=0.018, median 173 pg/ml vs. 0 pg/ml), and mRNA expression (p=0.012, median 42 RU vs. 3 RU) in activated PBMC from patients with T1D. Also ROR-C2 (p=0.02, median 70 RU vs 19 RU), IL-22 (p=0.005, median 3.6 RU vs. 0.9 RU), and FOXP3 (p=0.03, median 40 RU vs. 25 RU) showed increased expression in diabetic children. No difference was observed in the expression level of IFN-γ or T-bet. IL-17A and IL-22 mRNA was expressed in the population of CD4+ memory T-cells from children with T1D, whereas minimal or undetectable expression levels were seen in healthy children (6/8 vs 0/8; p=0.007 for IL-17 and 5/8 vs 0/7; p= 0.026 for IL-22). Higher expression level of FOXP3 in CD4+ memory T cells were seen in children with T1D than in healthy children (5/9 vs 0/8; p=0.009). IL-17 positive T cells of T1D patients appeared to be CD4+ cells expressing TCR-αβ and CCR6, and a subpopulation showed co-production of IFN-γ. In combination with IL-1β and IFN-γ, IL-17 enhanced up-regulation of iNOS, COX-2, SOD-2 and down-regulation of anti-apoptotic gene BCL-2.
Conclusion: The activation of IL-17/IL-22 immunity is a major immune alteration in children with T1D. Simultaneous up-regulation of IL-17 with FOXP3 or IFN-γ suggests aberrant immune regulation in T1D. IL-17 was shown to be detrimental for human islet cells, and IL-17 together with IL-1β and IFN-γ enhanced the stress response of islet cells. We conclude that the activation of IL-17 producing cells may be involved in the pathogenesis of human T1D.
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The role of regulatory $CD4^+CD25^+$ T lymphocytes and FoxP3 expression in evolution and progression of type 1 diabetes

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Background and aims: $CD4^+25^+$ T-lymphocytes (Treg) play a crucial role in the regulation of immune response. Lack of amount and/or activity may cause loss of self-tolerance to beta-cell autoantigens and the development of autoimmune process. The aim was to evaluate changes in terms of number or function of Tregs in high-risk diabetes subjects who are positive for diabetes-

specific autoantibodies and have a genetic susceptibility and type 1 diabetes mellitus (T1DM) patients at different stages of the disease.
Materials and methods: We examined 85 patients with T1DM at different stages of the disease: 36 patients with recent-onset T1DM, 12 - with the duration of diabetes from 1 to 5 years, 11 - from 5 to 10 years, 26 - over 10 years. 14 subjects at high-risk diabetes and 8 healthy subjects (control group) also included in the study. The HLA-DR and DQ alleles were detected by using PCR method. $CD3^+$, $CD4^+$, $CD8^+$, $CD38^+$, HLA-DR^+, $CD25^+$, $CD4^+25^+$ cells were analyzed by flow cytometry. FoxP3 expression was determined by Real Time PCR. All subjects were tested for islet cell antibodies, autoantibodies to glutamic acid decarboxylase, insulin and tyrosine phosphatase, C-peptide, HbA_{1c}.
Results: An increasing tendency of CD 25^+ and $CD4^+25^+$ T-lymphocytes and decreasing tendency of FoxP3 expression between control and high-risk subjects was observed ($p<0.1$). Significant differences were found in content of activation molecules CD38 and HLA-DR ($p<0.05$) in those groups, indicating the tension of the immune system. There was no significant difference in the percentage of Tregs in patients with recent-onset T1DM (0.8% [0.6; 0.8]) and control subjects (0.8% [0.3; 2.1]). However FoxP3 expression was significantly lower in patients with recent-onset T1DM than in the control group (0.39 [0.18; 0.71] vs. 1.11 [0.66; 2.26], $Z = 5.26$, $p<0.001$). Reduced FoxP3 expression was observed at any stage of the disease compared with control subjects. FoxP3 expression was 0.37 [0.18; 0.89] ($Z = 2.62$, $p<0.01$) in patients with duration of diabetes from 1 to 5 years; 0.14 [0.11; 0.31] ($Z = 3.33$, $p<0.01$) in patients with duration of diabetes from 6 to 10 years and 0.47 [0.17; 0.86] ($Z = 3.15$, $p<0.01$) over 10 years of the disease.
Conclusion: A rising tendency of Tregs in high-risk subjects may appoint for suppression of autoimmune process. Despite the fact that there was no significant difference in the content of Tregs in patients with T1DM compared with control subjects, their functional activity - FoxP3 expression was significantly lower at any stage of the disease, which may indicate the reduced ability to suppress T cell proliferation.

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The deficiencies of T regulatory lymphocytes (Tregs) in cell amount, expression and coordination of suppresion-related proteins at type 1 diabetes onset are only partially remedied in long term patients

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Background and aims: T regulatory lymphocytes (Tregs) play an important role in tolerance and autoimmunity. Tregs, defined as $CD4^+CD25^{high}$ T lymphocytes, and are thought to regulate the immune response in mammals. We previously showed a deficiency of Tregs in cell amount, expression and coordination of suppression-related proteins in newly-diagnosed type 1 diabetics (t1d) compared to controls. The aim of our study was to investigate the various features of Tregs in long standing t1d patients and to identify differences between them and newly diagnosed t1d patients.
Materials and methods: Peripheral blood from 13 newly-diagnosed patients (9M/4F, ages 12.5 ± 9.4 years), 26 long-standing patients (12 M/14 F, ages 26.7 ± 9.2 years) with mean disease duration of 11.6 ± 7.4 years (range 2-25 years) and 32 healthy controls with no first or second degree relatives suffering from any autoimmune disease (13 M/19 F, ages 25.3 ± 11 years) was analysed by flow cytometry for various phenotypic markers of Tregs. We included as such, molecules that had been linked to Treg function (FoxP3, CD28, CD45RO, CD127, CD152, TGFβ and TGFβRII), gene products or their receptors linked to heredity in type 1 diabetes (HLA-DR/DQ, CD25, InsR, CD152) and proteins linked to apoptosis (CD95) and cell proliferation (CD27). The statistical analysis was performed using SPSS for Windows, Version 16.0.
Results: Newly-diagnosed t1d patients have a significantly lower percent of Tregs (as percent of total $CD4^+$ T cells) compared to controls: 1.259 ± 0.264 % vs 3.047 ± 0.264 % respectively, $p < 0.001$. Long standing t1d patients have an intermediate value of Tregs, 1.748 ± 0.308 %, that is significantly different from that of both controls ($p < 0.001$) and long standing patients ($p < 0.001$), suggesting a "rebound phenomenon". Tregs of controls exhibit highly coordinated control of the frequency of expression of markers such as IL-2Rβ, CTLA-4, InsR, TGFβ, TGFβRII, HLA-DQ, and HLA-DR, several of these being products of t1d susceptibility genes. In newly-diagnosed patients there is a near total absence of coordinated expression of these markers, and a decreased level of expression of TGFβ and TGFβRII. In long-term patients there is a deficiency of expression of TGFβ, while there is an increase in the level of TGFβRII and the frequency of Tregs expressing HLA-DR and CD95; here the coordinated expression involves mostly CD45RO, CD95, IL-2Rβ, and HLA-DQ.
Conclusion: Our data are consistent with a pathogenesis mechanism that actively disrupts the amount, functioning and coordinated expression of effector molecules in Tregs at disease onset, while at long term these deficiencies are only partially remedied.
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The effect of Vitamin D supplementation on peripheral regulatory T cells in healthy humans, a randomised controlled trial

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Background and aims: Regulatory T cells (Tregs) play a central role in the maintenance of self tolerance and immune homeostasis. Thus an imbalance between different types of T cells, such as effector T cells and Tregs, has been reported to play a major role in the pathogenesis in autoimmune diseases like type 1 diabetes. Growing bodies of evidence from animal and in vitro studies suggest a role for vitamin D in modulating the function of CD4+ T cells, including Tregs. In the present study, we aimed to elucidate, whether supplementation with vitamin D has the potential to increase the number or function of peripheral Tregs in apparently healthy human individuals. Furthermore we investigated the pleiotropic in vivo effects of Vitamin D supplementation on other important circulating cells of the human innate and adaptive immune system.
Materials and methods: A double-blinded and placebo controlled trial was performed among 35 healthy subjects (46% females, mean age: 35 ± 11 years). Subjects were randomized to oral vitamin D (140 000 IU monthly) or placebo treatment for 3 months. Peripheral blood was drawn at baseline and monthly visits and the percentage of Tregs within 20000 CD4+ T cells as well as the percentage of other circulating cells of the human innate and adaptive immune system were determined by using a multi-parametric FACS-analysis. Functional tests for FACS-sorted Tregs were assessed in a 3H-Thymidin based suppression co-culture, including stimulation of effector cells with anti CD3/CD28 beads.
Results: 25-hydroxyvitamin D levels were significant higher in the vitamin D treated group than in the placebo group (mean level at 3 months: 60.2 ± 21.1 ng/ml vs 18.8 ± 7.2 ng/ml). Accordingly, the median percentage of Tregs increased significantly from a median baseline level of 4.8% (interquartile range: 4.3-5.8%) to a level of 6.4% (5.9-7.2%) at month 3 ($p<0.001$ for general linear model with repeated measurements) in the vitamin D group whereas there was no statistically significant change in the placebo group ($p=0.956$). Between group comparison at month 3 revealed a significant difference in the median levels of %Tregs ($p=0.017$). Suppressive function of regulatory T cells remained unchanged in both groups. Furthermore, analysing the other peripheral immune cells showed no statistically significant chances in the frequency of naive CD4+ and CD8+ T cells, memory CD4+ and CD8+ T cells, CD25++CD8+ cells, NK cells, NKT cells, B cells, monocytes, granulocytes, stem cells and blood dendritic cells (myeloid and plasmacytoid DC) in both groups. C-reactive protein was within the normal range before and after the treatment and did not change significantly between the study visits. In both groups no clinically relevant adverse events have been reported.
Conclusion: High dose of vitamin D supplementation increased the frequency of peripheral Tregs significantly in healthy subjects. This finding supports previously described associations of vitamin D deficiency and autoimmune diabetes and provides a rationale for further studies to investigate the immunemodulatory effects of vitamin D in diabetic subjects.

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Insulin treatment during pregnancy induces insulin-reactive CD4+CD25+FOXP3+ regulatory T cells in the infant

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Background and aims: Reduced risk for type 1 diabetes (T1D) has been reported in the offspring of mothers with T1D when compared to children of affected fathers. To evaluate the hypothesis that exposure of the offspring to maternal diabetes and insulin therapy results in tolerization to insulin *in utero* and thereby decreases the risk for T1D, we compared the FOXP3 expressing regulatory T cells in cord blood of infants born to mothers with or without T1D.
Subjects and methods: Cord blood mononuclear cells from 20 infants with maternal T1D and from 20 infants with an unaffected mother were analysed for the expression of CD4+CD25+ cells and FOXP3 ex vivo and after 72h stimulation with human insulin by flow cytometry. The mRNA expression of FOXP3, TGF-β and IL-10 was measured by real-time RT-PCR.
Results: The percentage of CD4+CD25^{high}FOXP3+ cells in cord blood was higher in the infants of mothers with T1D than in the infants of unaffected mothers ($p = 0.023$; median values 31.5% [range 6.1-63.1%] and 16.4% [range 2.0-36.3%], respectively). The numbers of FOXP3 positive CD4+CD25^{high} cells were higher after *in vitro* stimulation of cord blood cells with human insulin than in unstimulated cord blood cells in the infants with maternal T1D ($p < 0.001$; Wilcoxon test; median values 17.2% [range 2.4-56.9%] and 28.1% [range 8.4-60.8%]). In infants of non-diabetic mothers there was no difference in FOXP3 expression in CD4+CD25^{high} T cells stimulated with insulin compared to non-stimulated CB mononuclear cells ($p = 0.21$, Wilcoxon test; median values 10.9% [range 1.0-35.3] and 15% [range 0.7-31.4%]. Furthermore, an increased intensity of FOXP3 in CD4+CD25^{high} cells was observed in response to insulin stimulation in infants of T1D mothers ($p < 0.05$). Only in infants with maternal diabetes FOXP3-, IL-10- and TGF-β specific mRNA increased in cord blood cells in response to insulin (Wilcoxon- test $p < 0.001$, $p = 0.003$, $p = 0.013$, respectively). The HLA genotype did not modulate the results.
Conclusion: We suggest that maternal insulin treatment induces expansion of insulin-specific FOXP3 expressing regulatory T cells in the fetus, likely due to tolerization to insulin through transplacental transfer of insulin bound to insulin antibody complexes.
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Otelixizumab differentially modulates human regulatory and non-regulatory T cells

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Background and aims: Otelixizumab is an Fc-disabled monoclonal antibody (mAb) with specificity for CD3ε that has been shown to preserve β cell function in subjects with new onset type 1 diabetes mellitus (NOT1DM). Otelixizumab is being evaluated in placebo-controlled Phase 3 clinical studies in NOT1DM patients. The cellular and molecular mechanisms by which otelixizumab and other anti-CD3 mAbs turn off the autoimmune attack in humans are not well understood. Although otelixizumab binds to all T cells, treated subjects do not exhibit signs of chronic immunomodulation other than arrest of the pathogenesis mediated by pancreas-specific T cells. This suggests that this apparently non-antigen-specific therapy selectively signals antigen-activated T cells. Furthermore, peripheral blood Foxp3$^+$ CD4$^+$ regulatory T cells (Tregs) increase after otelixizumab administration, suggesting that it promotes Treg expansion/migration as well as function. This prompted us to investigate whether otelixizumab has a differential effect on distinct T cell subsets and whether it preferentially regulates activated T cells in an *in vitro* model of antigen reactivity.
Materials and methods: Mixed lymphocyte reactions (MLRs), using HLA-A2 mismatched donors to distinguish responders from stimulator cells, were conducted without or with otelixizumab. Proliferation and survival of CFSE-labeled responder Tregs, activated effector T cells (Teffs), and naïve T cells were examined by flow cytometry.
Results: Otelixizumab markedly inhibited the proliferation of alloantigen-specific Teffs in primary MLRs as well as secondary MLRs, where the mAb was added to primed alloantigen-specific Teffs. Inhibition of T cell proliferation was dose-dependent and peaked at the highest saturating concentration of otelixizumab. Subsaturating concentrations of mAb resulted in the proliferation of a subset of T cells with an activated but not a naïve phenotype, and the number of naïve T cells did not decrease in the presence of mAb. Addition of otelixizumab to primary MLRs was accompanied by the appearance of and an increase in the number of dividing Tregs, irrespective of the dose. Otelixizumab-induction of Treg proliferation was optimal at subsaturating concentrations. The dose-dependent inhibition of T cell proliferation was more pronounced among Teffs than Tregs, resulting in a ~5-fold decrease in the ratio of dividing Teff:Tregs at saturating concentrations of mAb as compared to controls. Furthermore, addition of otelixizumab to primary MLRs was associated with increases in non-dividing Tregs, suggesting that otelixizumab can induce Treg-commitment in activated T cells. Lastly, no mAb-induced cell death was observed.
Conclusion: These studies indicate that Tregs, activated Teffs, and naïve T cells respond differently to otelixizumab and to different concentrations of otelixizumab. Naïve T cells were minimally affected by otelixizumab. In contrast, otelixizumab induced a significant increase in Tregs that exhibited a lower susceptibility to otelixizimab-mediated inhibition of proliferation than activated Teffs. This suggests that otelixizumab's immunoregulatory effects are principally confined to activated Teffs and Tregs, and are markedly affected by dose. Further experiments comparing otelixizumab-induced signaling cascades in the various T cell subsets and the antigen specificity and potency of otelixizumab-expanded Tregs are being conducted.

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Dynamic changes of CD95 and CD95L expression on lymphocytes in patient with new-onset type 1 diabetes mellitus

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Background and aims: The CD95 molecule triggers cell apoptosis. It is expressed by activated lymphocytes and involved in switching-off the immune response. Impaired CD95 expression may play a role in type 1 diabetes mellitus (T1DM) initiation. Expression of apoptosis molecules is impaired in T1DM. Aims were: (i) To determine expression of apoptosis molecules on lymphocytes in patients with T1DM at onset of disease. (ii) To compare these parameters during the first year of the disease.
Materials and methods: 29 patients (12 women and 17 men) T1DM included. Age of onset diabetes (median) was 29 years old. Expression of surface CD95 and CD95L molecules on peripheral blood lymphocytes was measured in onset, remission period, and 12 months after diagnosis. CD95 and CD95L expression was detected by flow cytometry, using monoclonal antibodies. Samples for HbA_{1c} analysis were collected at onset and after 3, 6 and 12 months. The patients were also examined for C-peptide, HLA-typing and tested for islet cell antibodies, antibodies to glutamic acid decarboxylase, insulin autoantibodies and tyrosine phosphatase-like IA-2.
Results: 17 (59%) patients entered partial remission and 3 (10%) patients had complete remission. The susceptible genetic haplotypes, in particular DR3/DR4, were prevalent. HbA_{1c} level was 11.5% [10.8; 12.9] at onset, 6.9% [6.5; 8.1] after 6 months and 6.5% [5.3; 7.9] after 12 months. CD95 expression on lymphocytes was significantly lower at onset T1DM than at remission of the disease (p=0.02) and than after 12 months (p=0.05). The amount of CD95-positive cells was 29% [27.1; 32.5] at onset; 33.5% [31.5; 38.8] at remission phase and 33% [29.5; 39.1] after 12 months. No significant differences were found in CD 95L expression on lymphocytes between onset and after 12 months (p=0,21). Expression CD95L was similar during first year of disease. The amount of CD95L-positive cells were 1.4% [1.0; 3.0] at onset; 2.5% [1.6; 5.2] 12 months later. Autoantibodies titers decreased in the remission phase of the disease.
Conclusion: There are a lower level of CD95 expression on lymphocytes in new-onset T1DM patient and increase of it during the first year of the disease. This low CD95 expression on lymphocytes could be a contributing factor to markedly decreased suppressive potential of these cells in the initial phase of disease. We suggest that suppression of activated lymphocytes apoptosis may contribute to prolongation of autoimmune response in new-onset T1DM.

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Analysis of expression of Th1 and Th2-associated chemokine receptors on CD4+ T lymphocytes: comparison between recent onset type 1 diabetics and nondiabetic first degree relatives

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Background and aims: Previous studies have reported an important role of the chemokine receptors CXCR3 and CCR4, which are associated with Th1 and Th2 CD4+ cell subsets respectively and involved in their extravasations into inflamed pancreatic islets, in the initial phase of Type 1 diabetes (T1D). However, the changes in CXCR3+ and CCR4+ subsets of the memory $CD4^+$ CD45RO+ T cells in recent onset type 1 diabetics and their nondiabetic first-degree relatives (FDRs), have not yet been clarified. Therefore, the aim of this study was to analyze the percentage of the (a) CXCR3+ (Th1 associated) and (b) CCR4+ (Th2 associated) subsets of T memory cells, in peripheral blood in 24 recent-onset T1D patients in insulin-requiring state (IRS) at the onset (group A), 10 T1D patients in the state of clinical remission (CR) (group B), 41 nondiabetic FDRs (group C), as well as in 18 healthy, age-matched control subjects (group D).
Materials and methods: T1D was diagnosed in accordance to WHO criteria. The CR was defined as optimal metabolic control without insulin lasting >30 days. The percentages of CXCR3+ and CCR4+ T memory $CD4^+$ CD45RO+ cell subsets were analyzed in peripheral blood by using four-color immunofluorescence staining and flow cytometry.
Results: We found that there was no difference among the groups concerning the percentage of CD4+CD45RO+ T lymphocytes (A: 27.23±8.54 vs B: 24.19±6.50 vs C: 25.73±6.78 vs D:27.67±6.59 %, A vs B vs C vs D, p=NS). However, when the percentage of CXCR3+ T memory lymphocytes was analyzed, we found that in groups A and B it was significantly lower than in groups C and D (A: 40.19±11.52; B: 42.16±11.13; C: 53.92±8.19; D: 53.09±6.29 %; A vs C, D: $p<0.001$; B, vs C, D: $p<0.01$), while there was no difference between groups C and D. Simultaneously, the percentage of CCR4+T memory cells was also found to be significantly lower in groups A and B than in groups C and D (A: 31.53±9.67; B: 31.40±8.14; C: 39.88±9.10; D:40.90±7.24%; A vs C, D and B vs C,D $p<0.01$) also without difference between goups C and D.
Conclusion: Our results have shown that the onset of T1D was associated with the decreases in the CXCR3+ and CCR4+ subsets of T memory lymphocytes, presumably reflecting their extravasation into pancreatic tissue. However, these changes could not be detected in the nondiabetic FDRs, thus implying that the onset of the disease could be modified on the level of these subsets of T memory cells.

PS 23 Inflammatory mediator responses and markers in type 1 diabetes

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Systemic cytokines and chemokines in prediction of type 1 diabetes in high risk antibody positive subjects

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Background and aims: Type 1 diabetes is the result of a chronic immune mediated destruction of ß-cells lasting several months to years before manifestation of overt hyperglycemia. Cytokines and chemokines have been shown to orchestrate the immune response and can also directly harm ß-cells and associate with the disease process after diabetes manifestation. This study therefore aimed to evaluate whether systemic concentrations of cytokines and chemokines predict type 1 diabetes.
Materials and methods: Serum samples from baseline visits of 291 first-degree ICA-positive relatives, (55% males / 45% females, age 20±6.1years) of the European Nicotinamide Diabetes Intervention Trial (ENDIT) were analysed for chemokines (IL-8, MIP1-α) and cytokines (MIF, TNF-α, interleukin (IL)-13, IL-1ß, IL-1receptor antagonist [IL-1ra]). The clinical endpoint (overt diabetes mellitus) was monitored during the observational period of 5 years. Subjects were grouped into two age groups, below and above 16 years of age, as disease progression is thought to be faster in younger versus older subjects. Immune-mediators were measured by ELISA based technology. Regression analysis was adjusted for potential confounders sex, age, body mass index (BMI) and nicotinamide treatment.
Results: 73 (25%) subjects were diagnosed with type 1 diabetes during the follow up, with the earliest diabetes manifestation observed after one year and the latest after 5 years. 218 (75%) subjects remained non-diabetic. As known from previous ENDIT analyses, diabetes developed more frequently in pediatric (56 of 138, 41%) than in adults (16 of 153, 10%; p=0.001). Subjects above age 16 showed higher IL1ra concentrations compared to children (239 pg/ml versus 183 pg/ml, p=0.0197). All other immune parameters did not differ between pediatric versus adult subjects. Within the age groups, neither IL1ra, nor IL-8, MIP1-α, MIF, TNF-α, IL-13 or IL-1ß were predictive for later diabetes development.
Conclusion: In our study systemic chemokines, cytokines or cytokine receptor antagonist concentrations obtained one to five years before diabetes onset did not serve as predicitive markers for disease manifestation. The age-associated increase of IL1ra in adult subjects may contribute to the overall reduced rate of diabetes development compared to younger subjects.

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Proinflammatory and regulatory cytokines are similar in type 1 diabetes (T1D) and LADA and lower compared to type 2 diabetes (T2D) patients: results from Action LADA Study

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Background and aims: LADA (latent autoimmune diabetes in adults) is clinically almost indistinguishable from T2D but has T1D like islet antibodies (GADA). Previous studies showed that cytokines are involved in the pathogenesis of T1D and T2D. As cytokines play an important role in pathogenesis

of T1D and T2D we aimed to search for differences of LADA compared to T1D and T2D.
Material and methods: We investigated systemic cytokines of 90 patients with T1D (F/M= 28/62, age 45±10yrs), 61 LADA patients (35/26, 52±10yrs) positive for GADA who had not required insulin for at least 6 months after diagnosis, 465 T2D patients who were GADA negative (202/263, 55±9yrs) and 41 control subjects (C) (25/16, 47±9yrs). All patients were recruited from the Action LADA cohort with diabetes duration <5yrs. Anti-inflammatory IL-1ra and pro-inflammatory TNF-a and IL-6 from serum samples were measured by multiplex technology. Differences of circulating cytokine concentrations between all groups were assessed using Kruskal-Wallis test followed by Mann-Whitney test. With multiple linear regression models we compared log-transformed systemic cytokine concentrations of different groups as dependent variables using age, sex, BMI, blood pressure (BP) and diabetes duration as covariates.
Results: For all three cytokines significant differences were detected between different groups (all p<0.0001). Group by group comparison revealed similar cytokine concentrations in T1D and LADA patients. Compared with controls, T1D and LADA had significantly up-regulated IL-1ra, IL-6 and TNF-a (p<0.05). When comparing T1D and LADA versus T2D, all three cytokines were significantly higher in T2D (IL-6 p<0.001, TNF-a and IL-1ra p<0.05). As groups differed by BMI, age, sex, BP and disease duration we performed stepwise regression analysis with adjustment for these potential confounders. As expected, systemic concentrations of IL-1ra and IL-6 were positively associated with BMI (p<0.0001). TNF-a did not show an association with BMI. The significant increase of cytokines in T2D versus T1D/ LADA and C found in unadjusted comparisons persisted upon adjustment for sex, age, BMI, BP and diabetes duration in multiple linear regression models (all p<0.05).
Conclusion: We conclude that proinflammatory as well as anti-inflammatory cytokines are elevated in T2D compared to T1D, LADA and C. IL-1ra, IL-6 and TNF-a of LADA were lower compared to T2D but were similar compared to T1D. As cytokines cannot distinguish T1D from LADA, other factors are likely to determine clinical outcome that is characterized in immediate insulin need in T1D and preserved endogenous insulin secretion in LADA. Further studies should investigate which other factors might influence the pathogenic process leading to slower deterioration of ß-cell function in LADA and faster deterioration of ß-cell function in T1D.

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Serum CXCL1 levels are elevated in subjects with type 1 diabetes mellitus and possibly reflect the rate of c-peptide loss.
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Background and aims: Identification of unique inflammatory biomarkers may improve prediction of type 1 diabetes (T1D), and be used as clinical measures in trials aimed at preventing this disease. We previously compared transcript profiles of bone marrow (BM)-derived dendritic cells (DC) from NOD mice, a model of T1D, with those from a sister strain, NON mice, to characterise molecular changes in BMDC, and then found that BMDC from 4-week-old female NOD displayed 3-5 times stronger expression of inflammatory mediators, including CCL7, CXCL1, CXCL5, and S100A8/100A9, than those from NON. Intriguingly, in the human, these chemokines (Wang X, 2008) and S100 proteins (Collins CD, 2006) have been noted as possible biomarkers reflective of active anti-islet autoimmunity by microarray analyses. In the present study, we investigated weather serum levels of these molecules serve as effective biomarkers for T1DM, and those reflecting active autoimmune process.
Materials and methods: CCL7, CXCL1, CXCL5, and S100A8/100A were quantified by ELISA in sera from Japanese subjects with T1D and T2D as a matched disease control. Serum fasting C-peptide (CPR) levels were measured by CLEIA at the beginning of the study, and followed up after a year. The study groups consisted of: (i) 26 subjects (11 females, 15 males; median age 43 years, range 16-75 years; duration of diabetes 10 years, range 1-40 years) with acute-onset T1D; (ii) 20 subjects (10 female, 10 males; age 69 years, range 38-79 years; duration of diabetes age 10 years, range 3-26 years) with slowly progressive T1D; and (iii) 20 subjects (8 females, 12 males; mean age 10 years, range 21-83 years; duration of diabetes age 10 years, range 1-32 years) with T2D. All T1D subjects were positive for autoantibodies.
Results: It was noted that serum CXCL1 levels were significantly higher in subjects with acute-onset (median 113.2 ng/ml, range 41.75-457.2 ng/ml), and slowly progressive (median 100.8 ng/ml, range 32.87-225.0 ng/ml) T1D than in those with T2D (median 71.58 ng/ml, range 32.45-152.6 ng/ml, p=0.01 and 0.03, respectively, Mann-Whitney U test). Decreases in fasting CPR levels per year were significantly correlated with the CXCL1 levels (n=10, r=-0.01, p=0.02) in the subpopulation of slowly progressive T1D subjects displaying preserved beta-cell function, whereas cross-sectional fasting CPR levels were not. Serum CCL7, CXCL5, and S100A8/100A9 concentrations showed no difference between T1D and T2D subjects.
Conclusion: To our knowledge, this is the first report that reveals T1D subjects display elevated serum CXCL1 levels, irrespective of the subtypes, compared to control T2D subjects. CXCL1 is produced by monocytes and dendritic cells, as well as by beta cells, and reportedly involved in activating Th17 cells which play a pivotal role in developing T1D. The serum concentrations of this chemokine were able to predict the loss of CPR in slowly progressive T1D subjects, possibly reflecting activity of the anti-islet autoimmune process, however, failed to distinguish subtypes of T1D. The predictive value of CXCL1 in the development of acute-onset T1D should be further investigated in the first-degree relatives at risk or the recent onset subjects. In conclusion, we propose that the elevated serum CXCL1 levels are good markers for T1D, predisposing to anti-islet autoimmunity.

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High risk vs low risk nondiabetic first degree relatives of type 1 diabetics: differences in expression of Th1 and Th2 associated chemokine receptors and chemokine levels
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Background and aims: It has been previously suggested that expression of Th1 and Th2 associated chemokine receptors determine the recruitment of CD4+T cells into sites of inflammation during the onset of Type 1 diabetes (T1D). However, the relevance of the changes in expression of chemokine receptors, CXCR3+ (Th1 associated) and CCR4+ (Th2 associated), on T memory CD4+CD45RO+ cells and in respective chemokine levels, interferon-γ inducible chemokine (IP-10) (Th1 associated), and thymus-and activation-regulated chemokine (TARC) (Th1 associated), for the development of T1D has not yet been elucidated. Therefore, the aim of this study was to compare the changes in (a) percentage of CXCR3+ and CCR4+ subsets of CD4+ T memory cells (b) the level of chemokines IP-10 and TARC, in peripheral blood between two groups, the high-risk and the low-risk group, of nondiabetic first-degree relatives (FDRs) of patients with T1D as well as in the group of healthy controls. The difference between the two groups of FDRs was based on presence or absence of glutamic acid decarboxylase (GADA) and tyrosine phosphatase insulinoma antigen-2 (IA-2) antibodies. Thus, in the study we included 10 high-risk nondiabetic FDRs (GADA+, IA-2+) (group A) and 34 low-risk nondiabetic FDRs (GADA-, IA-2-) (group B) and 18 healthy unrelated control subjects (GADA-, IA-2-) (group C).
Materials and methods: T1D and glucose intolerance were excluded in the study by using WHO criteria. IP-10, TARC, GADA and IA-2 levels were determined by ELISA. The percentages of CD4+CXCR3+ and CD4+CCR4+ T memory cell subsets were analyzed in peripheral blood by using four-color immunofluorescence staining and flow cytometry.
Results: When the percentage of CXCR3+ T memory cells was analyzed, it was found to be higher in group A vs groups B and C (A: 64.27±6.53 vs B: 51.79±6.79; C: 53.09±6.29%, p<0.05). Simultaneously, the level of IP-10 was higher in group A vs groups B and C (A: 154.56±112.99 vs B: 109.73±79.52; C: 85.24±19.82 pg/ml, p<0.05). In contrast, the percentage of CCR4+ T memory cells was significantly lower in group A vs groups B and C (A: 30.04±3.26 vs B 41.90±8.59; C: 40.90±7.24 %, p<0.05). However, when TARC level was tested, it was similar in group A in comparison to group B and did not differ significantly compared to group C (A: 406.66±171.44 vs B: 336.01±166.26; C: 236.88±89.19 pg/ml, p=NS).
Conclusion: Our results have demonstrated that high risk FDRs showed higher levels of CXCR3+ T cell subset and IP-10 chemokine, both associated with increased Th1 response, together with lower levels of CCR4+ Th2 cell subset. The results imply that in FDRs the risk for developing T1D might be strongly influenced by enhanced activity of Th1 and diminished activity of Th2 autoimmune response.

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The association between vitamin D levels and biomarkers in newly diagnosed children with diabetes and their siblings

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Background and aims: Vitamin D insufficiency has been suspected as a contributing factor to the development of type 1 diabetes. Different cells in the immune system are capable of converting 25-OH to its active form 1,25 OH and vitamin D has been shown to suppress Th1 cytokines and enhance Th2 cytokines. The aim of the present study was to investigate the association between levels of vitamin D and different cytokines and chemokines.
Materials and methods: Data is derived from *The Danish Diabetes Registry* (*DIA-REG B&U*) initiated in 1996 with an attached bio bank. All serum samples were analysed using high-capacity Luminex xMAP technology. Vitamin D is determined by HPLC and divided in quartiles. Data was analysed using multiple regression with biomarkers as outcome and vitamin D in quartiles and CYP27B1 genotype as explanatory variables together with age and gender. All children were genotyped for the CYP27B1 variant rs4646536.
Results: There were 466 newly diagnosed children with diabetes and 466 healthy siblings. 509 were males and 423 females. We found no difference in vitamin D level between patients and siblings. CXCL8 (0.34 +/- 0.12; p<0.001), IL-4 (0.12 +/- 0.04; p=0.01), IL-18 (0.03 +/- 0.028; p=0.03) and IL-1b (0.20 +/- 0.11; p<0.001) significantly increased with increasing vitamin D level in the joined analysis of patients and siblings, when only patients were analysed CXCL8 (0.20 +/- 0.17; p=0.02), IL-18 (0.07 +/- 0.04; p<0.001) and IL-1b (0.16 +/- 0.15; p=0.04) were still positively associated with vitamin D. No association was found with IL-10, IL-12, IFN-g and TGF-b. Only for IL-1b and IL-18 there was a significant association with CYP27B1 genotype
Conclusion: We found no indication of suppressed Th1 and enhanced Th2 in either patients or healthy siblings with high levels of vitamin D but on the contrary increasing levels of Th1 oriented cytokines/chemokine IL-1b, IL-8, CXCL8 and Th2 oriented cytokine IL-4 with each quartile in vitamin D. The effect of vitamin D in relation to onset may be questioned.
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MicroRNA profiling from peripheral blood of diabetic patients

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Background and aims: MicroRNAs (miRNAs) play important roles in post-transcriptional control of gene expression. While specific miRNAs are implicated in several disease processes including type 2 diabetes (DM), few have been evaluated and validated in humans. We therefore profiled the miRNA expression from the peripheral blood of type 2 DM patients.
Materials and methods: We compared 12 DM male subjects with 12 healthy male controls. They were well matched in age [mean(SD) 37.3 (7.1) yrs] and body mass index [mean(SD) 26.1 (5.6) kg/m^2]. Blood pressure (BP) and fasting blood for glucose, lipids and HbA1c were measured. Total RNA was extracted from peripheral blood for miRNA profiling and real-time PCR analysis. The signal log ratio and p-values were calculated. T-test was used to compare DM subjects with controls.
Results: Systolic BP, fasting glucose, HbA1c, total cholesterol and LDL-cholesterol levels were significantly higher in DM subjects compared with controls (all P<0.05). Compared with controls, we identified 37 differentially regulated miRNAs in DM subjects. Among them, 21 miRNAs were upregulated (two to five-fold change, P<0.01) and 16 miRNAs were downregulated (1.5 to two-fold change, P<0.01). These miRNAs are primarily involved in regulating pancreatic development and functions, adipocyte differentiation, insulin signaling and glucose-dependent insulin secretion.
Conclusion: We have found several differentially expressed miRNAs from the peripheral blood of DM patients that are involved in glucose homeostasis and related functions. These miRNAs can be used as markers of disease progression and may be developed for therapeutic applications in DM.
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Virus-induced beta cell death depends on CXCL10 and the AKT-JNK-PKR crosstalk

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Background and aims: Both, type 1 (T1DM) and type 2 diabetes (T2DM) result from β-cell destruction and decreased β-cell mass. Virus infection seems to be an important environmental factor for the development and progression of the disease. Group B coxsackievirus (CVB) infection of β-cells has been associated with the development of diabetes. Intra-islet viral particles have been detected in pancreata from patients with T1DM and T2DM, but the mechanisms of a correlation between virus infection and diabetes progression are poorly understood. In this study we asked the question whether virus induced cytokines and chemokines correlate with β-cell destruction and which intracellular signals are involved.
Materials and methods: Isolated human islets and the CM human β-cell line were infected with two different CVB serotypes B3 and B4. Replication of CVB was confirmed by immunostaining of viral protein 1 (VP1) and titration of islet lysates. CXCL10 secretion from the islets was measured by ELISA, CXCL10, IFNβ, IFNγ, IL-1β, TNFα, IL-6, MCP1 and IL-8 mRNA production by quantitative RT-PCR and β-cell apoptosis by double-staining for the TUNEL assay and insulin. Islet protein expression and phosphorylation were analyzed by western blot.
Results: CVB3 and CVB4 infection of isolated human islets and β-cells induced a high virus replication, VP1 positive staining in up to 70% of the β-cells and resulted in a 7-fold increase in β-cell apoptosis in both the CVB3 and -4 infected islets together with an abolished glucose stimulated insulin secretion. This correlated with the increase in CXCL10, IFNβ, IL-1β, TNFα, IL-6, MCP1 and IL-8 mRNA levels in the infected islets. CXCL10 was the highest induced factor (~35-fold increase in secretion, 15-fold increase in mRNA, p<0.001), compared to uninfected islets. In contrast, IFNγ remained unchanged, suggesting that CXCL10 upregulation was independent of the known induction pathway. To determine the host cell pathways involved in CVB infection, we analyzed the kinetics of pro- and anti-apoptotic signals. We have previously shown, that CXCL10 induces transient Akt phosphorylation despite massive induction of β-cell apoptosis. In line with this data, Akt was also activated during CVB infection within 30 minutes p.i. and lasted for up to 24 h. After 24 h, Akt was downregulated together with an up-regulation of phospho c-Jun NH2-terminal kinase (pJNK), activation of Caspase-3, strong induction of CXCL10 and the appearance of VP1, switching survival signals into apoptosis. A link of such pro-inflammatory signals and viral infections is provided by double-stranded RNA-dependent protein kinase R (pPKR). Both virus strains lead to phosphorylation of PKR as well as its substrate eIF2α in islets. Blocking PKR phosphorylation by 2-aminopurine lead to a decreased VP1 expression.
Conclusion: Our data show that CBV infections have a direct deleterious effect on β-cell survival, resulting from virus-induced activation of pro-inflammatory cytokines and chemokines. During the early phase of infection, the virus triggers the Akt pathway, possibly to initially maintain host survival and its own replication. Antagonism by JNK and PKR phosphorylation would switch β-cell pro-survival paths into death. Further understanding of the pathways involved in viral infection of β-cells will be of particular interest in order to develop new therapies to rescue the β-cell.
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Coxsackievirus B3 induces the mRNA expression of IFNbeta and viral recognition receptors in human pancreatic islets

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Background and aims: Many observations have suggested a role for enterovirus infections, especially those with Coxsackieviruses (CV), in the etiopathogenesis of type 1 diabetes (T1D). Enteroviruses may contribute to T1D development by inducing functional impairment of beta cell functions, and/or beta cell destruction. An important immune response upon infection is the rapid production of type 1 interferons (IFNs) that inhibit viral spread and facilitate viral clearance. Interferon production is initiated via viral recognition by pattern recognition receptors. Cellular detection of RNA viruses

is mainly dependent on two receptor families, the toll-like receptors (TLRs) including TLR3, 7 and 8, and the cytoplasmic retinoic acid-inducible gene I -like helicases (RLHs) including melanoma differentiation associated gene 5 (MDA5) and retinoic-acid-inducible protein I (RIG-I). Stimulation of these sensors trigger the production of pro-inflammatory cytokines and type I IFNs. The aim of this study was to investigate the expression of intracellular viral sensors and IFNβ in human pancreatic islet cells infected with CV.
Material and method: Human islets from 20 donors were obtained from the Nordic Network for Clinical Islet Transplantation. The mRNA expressions of IFNβ, MDA5, RIG-I, TLR3, 7 and 8 were measured in human islets 48 h after infection with CV serotype B3 (CVB3) using quantitative Real-Time PCR analysis.
Results: Human islets responded to a CVB3 infection by upregulating the mRNA expression of IFNβ ($p<0.0005$ vs. uninfected control). Moreover, there was a significant upregulation of the mRNA expression for the intracellular viral sensors MDA5, RIG-I and TLR3 ($p<0.01$, 0.005, 0.005, vs. uninfected control, respectively). A small, but not significant, upregulation of TLR7 and TLR8 mRNAs was also observed ($p=0.16$ and 0.21, vs. uninfected controls, respectively).
Conclusion: Our results show that human pancreatic islet cells express low basal levels of intracellular viral sensors and IFNβ, which are robustly induced upon CVB3 infection. Mounting an antiviral response rapidly upon infection might be important for beta cell survival and protection against virus induced type 1 diabetes.
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Role of STAT-1 and IRF-1 in cytokine-mediated dysfunction and apoptosis in mouse islets of Langerhans
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Background and aims: Type 1 diabetes (T1D) is an autoimmune disease characterized by a T-cell mediated destruction of the pancreatic beta cells. Cytokines, such as IL-1β and IFN-γ, play a crucial role in beta cell destruction, by activating two different pathways, NF-κB and JAK/STAT, among others. Previous work from our group discovered a dual role for the IFN-γ signaling pathway. If the pathway was interrupted at the level of STAT-1, islets and purified beta cells were fully protected against cytokine-induced apoptosis; however, deletion of the direct downstream effector gene of STAT-1, IRF-1, showed a dual response. Thus, whole mouse islets were partially protected *in vitro*, while purified beta cells demonstrated no difference in protection against cytokine-induced apoptosis, suggesting a role for non-beta cells in the islets in this destruction. *In vivo*, IRF-1 deletion in islets was associated with a higher prevalence of primary non-function and shorter functioning graft survival. In the present study, we aimed to study the protein profiles in islets with a disruption of either transcription factor exposed to inflammatory cytokines.
Materials and methods: Alterations in protein profile upon cytokine-treatment were investigated in islets from C57BL/6, STAT-$1^{-/-}$ or IRF-$1^{-/-}$ mice after 24h incubation with/without a combination of IFN-γ (1000 U/ml) and IL-1β (50 U/ml). Differential protein expression was analyzed using 2-dimensional difference gel electrophoresis (2D-DIGE) and differentially expressed spots were identified by MALDI-TOF/TOF. In parallel, susceptibility to apoptosis was measured by microscopic counting (Hoechst/PI staining).
Results: While the combined treatment of IL-1β + IFN-γ clearly caused apoptosis in C57BL/6 islets (40.6±3.7%, similar to 5.6±1.1% apoptosis for non-treated islets, $p<0.001$, $n=12$), STAT-$1^{-/-}$ islets were completely protected against cytokine-induced apoptosis (4.6±0.3% compared to 5.0±0.7% for non-treated islets ($n=10$)) and IRF-$1^{-/-}$ islets were partially protected (25.1±4.4% compared to 5.1±0.7% for non-treated islets ($p<0.001$, $n=9$)). 2D-DIGE analysis, revealed a total of 1853 spots, of which 301 were differentially expressed ($p<0.05$, $n=4$). Of these, we identified by now 144 spots. 60% of these were exclusively regulated in the C57BL/6 islets, with identified proteins belonging to different functional groups such as mRNA processing, insulin signaling, metabolism and folding of proteins. Treatment with cytokines generated far less response in the proteome of STAT-$1^{-/-}$ and IRF-$1^{-/-}$ islets. Moreover, the proteomic response generated in the knock-out mouse islets was indicative for unique effects of *Stat1* or *Irf1* gene disruption. An example is heat shock 70 kDa protein 9 (GRP75), which had markedly lower levels in IRF-$1^{-/-}$ islets and higher levels in STAT-$1^{-/-}$ islets, as compared to control C57BL/6 islets ($p<0.05$).
Conclusion: We identified proteins whose regulation was not only dependent on STAT-1 and/or IRF-1, but whose functionality can explain the difference in sensitivity of these islets to cytokine-induced cell death. These proteins could be important targets for clinical intervention in T1D, as disruption of the JAK/STAT pathway is not a feasible option for treatment or prevention of T1D.

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T cell function is released from the anti-inflammatory effects of adiponectin in type 1 diabetes (T1D): a potential link between insulin resistance (IR) and T1D
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Background and aims: IR is independently associated with the development of T1D, though the mechanism for this is not clear. Adiponectin is an insulin sensitising and anti-inflammatory adipokine whose serum levels are reduced with IR. We hypothesised that changes in adiponectin and its receptors may mediate the link between IR and T1D. We have previously shown that receptors for adiponectin, AdipoR1 & AdipoR2, are expressed by peripheral blood mononuclear cells (PBMC), and in particular by monocytes and CD11c+ CD1a+ dendritic cells (DC). We also found the monocytic expression of AdipoR1 & AdipoR2 is reduced by 45% ($p<0.01$) in patients with T1D, compared to matched healthy controls as well as subjects with insulin treated type 2 diabetes. In this present study, we determined the effect of adiponectin on DC-mediated T cell proliferation, as well as the functional significance of the reduced AdipoR expression in T1D.
Materials and methods: 10 subjects with T1D were compared against 10 age and BMI-matched healthy controls. AdipoR1 & AdipoR2 expression was measured by flow cytometry and qPCR. In serum-free conditions, we studied changes in the expression of CD80, CD86, HLA-DR, CD1a, DC-SIGN on monocyte-derived DC, following exposure to adiponectin. The stimulatory capacity of DC on T cells and whole PBMC proliferation was assessed by CFSE dilution.
Results: The addition of adiponectin to CD14+ monocytes decreased the expression of CD86 on DC at both RNA and protein level by 39-64%. This inhibitory effect on CD86 was dose dependent (IC50 = 1.3-1.9 ug/ml). This pronounced effect was not observed when adponectin was added at >24 hours of DC culture and was overcome by LPS added on day 5. The expression of CD1a, DC-SIGN, HLA-DR & CD80 did not alter. DC generated in the presence of adiponectin showed reduced stimulatory capacity when tested on CFSE+CD4+CD25- effector T cells. In T1D, adiponectin induced suppression of CD86 expression at the fixed dose of 10ug/ml was significantly decreased in T1D by up to 31% (IC50 = 1.55-2.08 ug/ml $p<0.05$). The degree of inhibition correlated with AdipoR1 protein expression of the monocyte precursors ($r=-0.69$ $p<0.05$). Furthermore, adiponectin treated DC from T1D subjects retained greater T cell stimulatory capacity, in the presence of OKT3. In corroboration, the suppression of PBMC proliferation by adiponectin to the common antigen tetanus toxoid was also significantly reduced in T1D (Healthy controls 48% vs 25% in T1D $p<0.05$).
Conclusion: T cells are released from the anti-inflammatory effects of adiponectin in patients with T1D. This mechanism may contribute to the association between IR and the development of T1D.
Supported by: NN

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Preservation of beta cell function by treatment with DiaPep277® - a retrospective analysis of phase II data in adult type 1 diabetes patients

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Background and aims: DiaPep277®, a peptide derived from the human Hsp60, modulates the immunological destruction of β cells that lead to autoimmune diabetes. Several phase II studies (352, 420, 441/451, 520) were conducted to evaluate the ability of DiaPep277® to preserve insulin secretion in adult type 1 diabetes (T1D) patients. Due to the small number of patients and the diversity in β cell function among T1D patients, a statistically significant difference could be demonstrated only for one study (420) despite the similarity in trend analysis in all 4 studies analyzed. To evaluate the efficacy of DiaPep277® in a larger number of patients, we performed a retrospective combined data meta analysis of the efficacy parameters.
Materials and methods: To compensate for the small group size in the individual studies, the efficacy data of studies 352, 420, 441/451, and 520 were pooled and analyzed. This is justifiable in view of the comparable demographic data. Patients were treated for 12 months and C-peptide levels were measured at month 13 and at month 18 (6 months after end of treatment, excluding study 441/451). Preservation of β cell function was evaluated as the change in total secreted C-peptide (AUC) following glucagon stimulation from Baseline to 13 and 18 months.
Results: A significant preservation of β cell function in patients treated with 1.0 mg DiaPep277® was demonstrated. At the end of treatment, the change in glucagon stimulated C-peptide AUC in the DiaPep277® treated group (n=58) was -0.36 nmol/l/min as compared to -2.79 nmol/l/min in the placebo group (n=46) (p=0.028). At the end of follow-up, although C-peptide values had declined in both arms, the decrease in the DiaPep277® arm was significantly smaller than that in the placebo arm, -2.28 ± 5.4 nmol/l/min versus -5.56 ± 7.0 nmol/l/min, respectively (p=0.04). This decrease may indicate that the treatment of adult patients should be longer than 1 year to maintain treatment effect. In addition, DiaPep277® treatment improved the ability to maintain the ADA-recommended HbA1c target of <7%. At the end of follow-up, 64% of DiaPep277® treated subjects reached the target glycemic level compared to only 47% in the placebo group.
Conclusion: The combined data analysis of the phase II studies in adult T1D patients showed a statistically significant treatment effect of preservation of β cell function and clinical benefit of improved glycemic control. This clearly indicates that the lack of conclusive difference in the individual studies stems from the small number of patients per arm and not from a limited treatment effect. Based on these results, 2 phase III studies are being conducted worldwide in newly diagnosed adult T1D patients to obtain regulatory approval for registration.

Phase II clinical studies in T1D subjects - Baseline demographic data (average ± SD)

	Study 352	Study 420	Study 441/451	Study 520
Gender	32 males 16 females	35 males	46 males 18 females	32 males 18 females
BMI	23.1±3.6	21.2±2.8	22.7±3.0	23.5±3.5
Age (years)	31±6.9	25.7±9.7	26±8.3	27.8±8.1
Basal C-peptide (nmol/l)	0.29±0.24	0.48±0.44	0.25±0.2	0.53±0.48
% HbA1c	5.93±0.92	6.83±1.27	7.61±1.57	7.02±1.36
Insulin Dose U/kg/day	0.44±0.17	0.34±0.18	0.34±0.23	0.37±0.20

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CoQ10 affects peripheral natural killer cells in patients with type 1 diabetes mellitus

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Background and aims: Natural killer (NK) cells have been implied in the pathogenesis of type 1 diabetes (T1D), although their exact role in the disease is still not known. T1D leads to long term complications, including increased mortality mainly due to cardiovascular disease (CVD). The antioxidant CoenzymeQ10 (CoQ10) has been suggested to decrease CVD risk by a mainly unknown mechanism. We here, for the first time, investigate the impact of CoQ10 treatment on the NK cells in T1D.
Materials and methods: Patient data and peripheral blood samples were collected immediately before and after 12 weeks of oral CoQ10 treatment (100 mg twice daily). Peripheral blood mononuclear cells (PBMC) were analysed using flow cytometry after staining with monoclonal antibodies. The NK cells were defined as $CD56^{+}CD3^{-}$ lymphocytes. NK cell frequency, subset distribution and phenotype in patients with T1D was compared with patients with type 2 (T2D) diabetes as controls since they too exhibit chronic hyperglycaemia, which by itself could influence lymphocyte phenotype and function, but lack the autoimmune pathology of particular interest to this study. 13 T1D and 10 T2D patients (mean age 58.1 ± 9.8 vs 62.8 ± 8.9 yrs, females 54 vs 30%, fasting plasma glucose 7.2±1.3 vs 6.9±3.3 mM p=0.27, HbA1c 6.7±1.3 vs 5.6 ±1.1, none significantly different) were treated 12 weeks with CoQ10. Retinopathy was more common in T1D (92 vs 40%, p= 0.02) while nephropathy was more common, although not significantly, in T2D patients (30 vs 8%). No difference was observed regarding neuropathy.
Results: Treatment with CoQ10 did not affect the overall frequency of NK cells in PBMC, in T1D or T2D patients. However, several phenotypic alterations of the peripheral pool of NK cells were observed after CoQ10 treatment. The NK cell activating receptor NKG2D has previously been shown to be downregulated in NK cells from T1D patients. Here we show that CoQ10 leads to a small but highly significant (p<0.001) upregulation of NKG2D on NK cells in T1D, hence potentially normalizing the NKG2D levels. Some NKG2D ligands are upregulated in response to stress and NKG2D has been implicated in several pathological conditions. We also observed an increased subset of CD11c expressing NK cells, both among the T1D and T2D (p<0.01 and p<0.05 respectively) after treatment. CD11c positive NK cells have been demonstrated to be potent cytokine producers and might interact in long term complications. NK cells are divided into two phenotypically and functionally distinct subsets on the basis of their expression level of the surface marker CD56. While NK cells with a bright expression of CD56 have a higher capacity to produce cytokines, the CD56 dim NK cells are more cytotoxic. In patient with T1D, we found a significant increase of CD56 bright NK cells after CoQ10 treatment. A similar trend was observed also in T2D. This finding further supports the idea that cytokine producing NK cells are increased in peripheral blood following CoQ10.
Conclusion: Our results demonstrate that CoQ10 treatment increased NK cells expressing CD11c in both T1D and T2D, as well as the percentage of CD56 bright NK cells and the activating receptor NKG2D in T1D. Collectively our results opens up for a possible role of NK cells in a CoQ10 mediated beneficial effect in patients with T1D.
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Does immune tolerance with Alum-GAD prevent or delay onset of type 1 diabetes in non-diabetic children with multiple islet autoantibodies?

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Background and aims: Type 1-diabetes (T1D) is predictable by HLA-risk genotypes and islet autoantibodies. The subclinical phase, lasting months to years, is defined by islet autoantibodies (A) against GAD65 (GADA), insulinoma-associated protein 2 (IA-2A), insulin (IAA) or the ZnT8 transporter (ZnT8A). A gradually deteriorating glucose metabolism precede clinical

onset. Children with genetic risk of T1D are followed prospectively by us in the Diabetes Prediction in Skåne (DiPiS) and The Environmental Determinants of Diabetes in the Young (TEDDY) studies. Immune tolerance with human recombinant GAD65 formulated with alum (Alum-GAD) has shown promising results to preserve residual beta-cell function in newly diagnosed T1D children. As only a fraction of the residual beta-cell function remains at onset, we initiated Diabetes Prevention -Immune Tolerance (DiAPREV-IT) as a first prevention study with Alum-GAD. The aim is to evaluate safety and efficacy of Alum-GAD in non-diabetic children from 4 years of age with multiple islet autoantibodies.

Materials and methods: DiAPREV-IT is an investigator-initiated, placebo-controlled, double-blinded study of Alum-GAD (donated by Diamyd Medical AB, Sweden) in children with GADA and at least one additional islet autoantibody. Children from 4 years of age (n=50) recruited from DiPiS and TEDDY will be treated with placebo (n=25) or Alum-GAD (n=25) in two doses of 20 microgram. The children are followed every 3rd month during the 5 year follow-up with alternating IVGTT and OGTT to evaluate beta-cell function. Placebo children developing T1D during the study period will be treated with Alum-GAD at clinical onset. GADA, IA-2A, IAA and ZnT8A (both ZnT8RA and ZnT8WA) are measured by standardised radioimmunoassays.

Results: As of April 1, 2010, a total of 26 islet autoantibody-positive children have been screened for participation. Of those, three children had lost GADA or the additional islet autoantibody at screening. One family withdrew their consent and another child was excluded due to illness. The included 21 children were 4-9.8 (mean 6.0) years old. All but one child (20/21) were positive for one or two of ZnT8R/WA and 17/21 were positive for IA-2A. While 2/21 children were positive for GADA and only one additional autoantibody, the remaining 19 children were positive for 3, 4 or 5 autoantibodies. A total of 17 children have received both injections, and an additional two children have received the first injection. The first visit after the second injection has been completed by 14 children. No serious adverse events were reported and none of the children have developed T1D. Mild to moderate injection site reactions were reported by all participants.

Conclusion: DIAPREV-IT is the first prevention study with Alum-GAD in non-diabetic children with multiple islet autoantibodies. In including analysis of the ZnT8RWA the number of T1D-risk children is increased. As no serious adverse events have been reported so far, DIAPREV-IT will continue to recruit children.

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Prediction of remission after 6 months and residual beta cell function 12 months after diagnosis in Danish children using multivariate statistical methods

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Background and aims: Children and adolescents with newly diagnosed type 1 diabetes (T1D) experience a remission period, where the requirement for exogenous insulin treatment declines or even becomes non-existent. The purpose of this prospective study, conducted in a Danish cohort of children with new onset T1D, is to identify patterns of early clinical and laboratory characteristics and to explore the metabolic disturbance and genetic background as predictors of the residual beta-cell function and remission the first 12 months after diagnosis.

Materials and methods: 129 children and adolescents aged < 17 years from 4 centres in Denmark with newly diagnosed T1D were followed for 12 months. HbA1c, autoantibodies, HLA typing and mixed meal stimulated C-peptide, proinsulin, Glp-1, GIP and Glucagon 1, 3, 6, 12 months after diagnosis were analyzed centrally. pH and standard bicarbonate were determined locally. In addition 40 SNPs in either T1D or T2D related genes have been genotyped. Regression models for prediction of log (C-peptide), and insulin dose adjusted HbA1c (IDAA1c) at 12 months was done by 10 fold cross validated Lasso for variable selection followed by Multiple Linear Regression (MLR) analysis on these relatively few selected variables. Covariates for age, gender and the clinical- and laboratory data were included in the analysis. Data is initially split randomly into 70% training data and 30% test data in order to estimate model performance from independent data. Statistical inference is reported for the test set.

Results: The best predictors for stimulated C-peptide concentration at 12 months were HbA1c at diagnosis (positive association (PA)), C-peptide at 1, 3, and 6 months (PA), IDAA1c at 3 months (negative association (NA)) and proinsulin at 6 months (PA). The selected variables are consistent over time, the model performance slightly improves when data are included over the different time points (r=0.74 0, 1 month data; r=0.79 0, 1, 3 months data and r=0.84 0, 1, 3, 6 months data) and all models are significant (p<0.001). Genotypes of common variants in the PTPN22 and TCF2 genes refine the C-peptide models, but they do not have a significant quantitative impact. HLA risk groups and autoantibodies were not selected by the models. The best predictors for remission after 6 months are C-peptide at 1 month (NA), stimulated blood glucose at 1 month (PA) and height at 1 month (NA), probably a surrogate marker for beta-cell mass. This model is significant (p=0.007, r=0.42) both when we use IDAA1c ≤ 9, to define partial remission (stimulated C-peptide > 300 pmol/l) and IDAA1c as a continuous variable (p=0.013, r=0.41).

Conclusion: This study illustrates the strength of multivariate statistical analysis when applied to observational data, in predicting the course of the residual beta-cell function and remission in children with new-onset T1D. The results suggest that disease progression and proportion of patients in remission can be predicted by objective factors present soon or later after diagnosis.

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Fasting and stimulated C-peptide at aseline in DEFEND-1, a phase 3 study of otelixizumab in new onset type 1 diabetes

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Background and aims: Stimulated C-peptide is frequently used to determine residual beta cell function. We compared mixed meal-stimulated and fasting C-peptide levels using baseline data from the DEFEND-1 Phase 3 study, and investigated the optimal single time point post-meal for determining maximum C-peptide in adults and adolescents with new onset type 1 diabetes mellitus (NOT1DM).

Materials and methods: DEFEND-1 is a multinational, placebo-controlled Phase 3 study of the safety and efficacy of otelixizumab, an investigational Fc-disabled anti-CD3 monoclonal antibody with T cell immunomodulatory activity, in subjects with NOT1DM (clinical trials identifier NCT00678886). Subjects were 12-45 years old, enrolled within 90 days of diagnosis, had BMI < 32, had at least 1 T1DM-associated autoantibody, and were otherwise healthy. Adult subjects had a mixed meal tolerance test (MMTT) with BOOST® at screening and another MMTT at predose (≤ 14 days before the first dose of study drug); subjects < 18 years had a single C-peptide assessment at 120 min post-meal at screening and a full MMTT at predose. To be eligible for DEFEND-1, subjects had to have a maximum stimulated C-peptide > 0.20 nmol/L at screening or predose. The current analysis includes data from one MMTT per subject: the predose MMTT if available, otherwise the screening MMTT.

Results: Data were available from 243 adult and 29 adolescent subjects who went on to receive either otelixizumab or placebo and 101 adults and 8 adolescents who failed screening. Baseline characteristics are shown in Table 1. Fasting and stimulated C-peptide values were available for 339 subjects. Fasting C-peptide was highly correlated with maximum stimulated C-peptide (r=0.76, p=<0.0001) and age group had no significant effect on this association. In adults, a fasting C-peptide > 0.1 nmol/L had a sensitivity of 88% and a specificity of 75% to identify subjects with a maximum stimulated C-peptide > 0.2 nmol/L. In adolescents, the same cutoff value had a sensitivity of 78% to identify subjects with a maximum stimulated C-peptide > 0.2 nmol/L; specificity could not be determined, as no adolescents had a maximum stimulated C-peptide < 0.2 nmol/L. Maximum stimulated C-peptide occurred at 120 min in most subjects (42%); maximum values occurred at 30, 60, and 90 min in 5%, 16%, and 35% of subjects, respectively. Of the 334 subjects with a recorded C-peptide at 120 min, 326 had a maximum stimulated C-peptide > 0.2 nmol/L; of these, only 2 (0.6%) had C-peptide < 0.2 nmol/L at 120 min.

Conclusion: In these subjects with NOT1DM, a fasting C-peptide level of > 0.1 nmol/L was a reasonable determinant of residual beta cell function and, when combined with a single 120 minute post-meal sample for subjects who fall below this threshold, captured 99% of subjects compared with a full

MMTT. These data are of relevance for future trials and interventions in this population.

Baseline characteristics of study population (data presented are means and SD unless specified)

	Enrolled adults, n=243	Screen ailed adults, n=101	Enrolled Adolescents, n=29	Screen failed adolescents, n=8
Age, Years	26.2 (5.85)	27.0 (6.59)	13.9 (1.78)	12.4 (5.21)
Female, n (%)	84 (34.6%)	28 (30.1%)	11 (37.9%)	2 (28.6%)
Caucasian, n (%)	231 (95.1%)	84 (83.2%)	24 (82.8%)	7 (87.5%)
Time since diagnosis, days	59.3 (19.34)	N/A	62.9 (18.5)	N/A
BMI	23.7 (3.12)	N/A	20.5 (2.58)	N/A
Insulin dose, U/kg/day	0.35 (0.19)	N/A	0.36 (0.16)	N/A
HbA1c, %	7.18 (1.35)	9.17 (2.43)	7.80 (1.49)	8.51 (1.82)
Max stimulated C-peptide, nmol/L	1.27 (0.77)	1.42 (1.27)	1.12 (0.68)	0.68 (0.35)
C-peptide AUC, nmol/L/min	0.90 (0.56)	1.06 (0.99)	0.85 (0.54)	0.47 (0.26)
Fasting C-peptide, nmol/L	0.31 (0.26)	0.47 (0.53)	0.31 (0.23)	0.17 (0.12)

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DIA-AID 1 - An international phase III clinical study to evaluate the biological effect of DiaPep277® in preservation of beta cell function in newly diagnosed T1D patients

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Background and aims: DiaPep277®, a peptide derived from the human Hsp60, modulates the immunological destruction of β cells that lead to autoimmune diabetes. Following phase II studies which demonstrated good safety and tolerability and preservation of β cell function after treatment with DiaPep277®, a phase III study was initiated and is currently being conducted in 40 medical centers in Europe, South Africa and Israel. The goal is to evaluate safety and therapeutic effect of DiaPep277® in preserving insulin secreting β cells in newly diagnosed type 1 diabetes (T1D) patients.
Materials and methods: The study is randomized, double blinded, placebo controlled. Major inclusion criteria: Age 16 - 45, diagnosed within 3 months, fasting C-peptide > 0.2 nmol/L and positive islet autoantibodies. Subjects received 1 mg DiaPep277® or placebo at 0, 1, 3, 6, 9, 12, 15, 18, and 21 months. Co-primary endpoints were defined as the change in total secreted C-peptide (AUC) measured by the glucagon stimulation test and by the mixed meal tolerance test. Secondary endpoints included glycemic control, daily dose insulin requirement and number of hypoglycemic events as determined by continuous glucose monitoring system. Clinical safety was monitored by electrocardiogram, blood chemistry and haematology. Immune monitoring included peptide-specific dermal sensitivity, antibody titers, cytokine and chemokine profiles and cell surface markers in a population sub-group.
Results: Out of 688 patients screened, 457 were randomized to the study. 221 patients have completed 2 years of therapy and 163 patients are still under treatment protocol. Screening failures were mostly due to fasting C-peptide levels being below the limit (18%) and to an absence of autoantibodies (14%). 16% of the randomized patients dropped out of the study, mostly because withdrawal of consent. Glycemic control was maintained during the treatment period. The mean value of HbA1c at baseline was 7.38% (n=457), at 12 months 7.2% (n=347) and at 24 months 7.46% (n=221). An independent committee that reviews the safety data every six months reported no drug-related safety concerns. Long term safety and treatment effect of DiaPep277® is being followed in an extension study in which patients are divided into a treatment arm and a follow-up arm.
Conclusion: Based on the efficacy and safety data from phase II studies, we designed a phase III clinical study in newly diagnosed T1D patients who are treated with 1 mg DiaPep277® for 2 years. Currently, 51% of the patients have completed 2 years of therapy. Safety evaluation indicates a very favorable safety profile. Results of the study are expected at the end of 2011. A second, confirmatory phase III clinical study is being initiated worldwide in newly diagnosed adult T1D patients.

Clinical and metabolic parameters of patients with T1D randomized into the study at baseline. Data is shown as average + SD and interquartile range (in brackets)

Age	27.15 ± 7.93 (20 - 32)
Gender	302 Males / 155 Females
BMI	24.08 ± 3.42 (21.6 - 26.09)
Fasting C-peptide	0.47 ± 0.25 (0.3 - 0.55)
%HbA1c	7.38 ± 1.7 (6.2 - 8.1)
Insulin U/kg/day	0.4 ± 0.24 (0.25 - 0.52)
Autoantibodies	IA2A+ve 60%, IAA+ve 74%, GADA+ve 86%

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Effect of sirolimus versus mycophenolate mofetil on glucose metabolism in pancreas and kidney transplantation: final results of a prospective randomised study

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Objectives: Metabolic effects of immunosuppressive agents are important in pancreas or islet transplantation. The aim of our study was to compare glucose metabolism in Type 1 diabetic pancreas and kidney recipients on tacrolimus-based immunosuppression in conjunction with sirolimus (RAPA) or mycophenolate mofetil (MMF) in a prospective randomised study.
Methods: The investigation was performed in 40 insulin-independent rejection-free patients after simultaneous pancreas and kidney transplantation (with systemic venous drainage of pancreatic graft) on discharge from the hospital (0.60±3 [mean±SD] month post-transplant; with steroid dose 11±4.3 mg/day) and subsequently at 21.2±9.9 months (steroid-free). All recipients had a good function of the kidney graft. Fasting glycemia, insulin and C-peptide levels, HbA_{1c}, IVGTT with K_G-calculation were assessed in both groups. Insulin sensitivity was evaluated by HOMA-IR. Areas under the insulin/C-peptide curves during the IVGTT (AUC-IRI, AUC-CP) were used as the parameters of insulin/C-peptide secretion.
Results: The RAPA and MMF groups did not differ in age, BMI, post-transplant period and steroid daily dose. Trough levels of tacrolimus had no significant impact on any of examined parameters. K_G and HOMA-IR of the whole study group significantly improved between the exams (1.0±0.4 vs. 1.43±0.44 %/min., p<0.001 and 4.1±4.1 vs. 2.73±1.98, p<0.05, respectively). We found only a significant difference between the groups in stimulated AUC-CP after steroid withdrawal.

	RAPA group (n=20)		MMF group (n=20)	
	Examination 1 (a)	Examination 2 (b)	Examination 1 (c)	Examination 2 (d)
HbA1c (%)	6.3±0.9	5.6±0.5 (p<0.01 vs. a)	5.9±0.6	5.4±0.6 (p<0.05 vs. c)
Fasting glycemia (mmol/L)	5.2±0.6	4.8±0.5 (p<0.05 vs. a)	5.4±0.8	4.7±0.7 (p<0.01 vs. c)
K_G (%/min.)	1.0±0.5	1.35±0.36 (p<0.01 vs. a)	1.0±0.3	1.51±0.5 (p<0.001 vs. c)
AUC-IRI (mIU/L/60min.)	2056±1321	2062±1229	3148±2270 **(p<0.01 vs. a)**	2601±1368
AUC-CP (pmol/mL/60min.)	138.7±57.3	96,6±29.8 (p=0.001 vs. a)	180±85,1	114,7±33,7 (p<0.01 vs. c)
Stimulated AUC-CP (pmol/mL/60min.)	47.9±24.7	47.6±18.8	73.8±29 **(p<0.01 vs. a)**	63.8±22.6 **(p<0.05 vs. b)**
HOMA-IR	3.4±2.7	2.6±1.7	4.7±5.0	2.8±2.2

Conclusion: Glucose tolerance measured by IVGTT significantly improved in whole study group probably due to better insulin sensitivity after steroid withdrawal. The higher stimulated C-peptide secretion in the MMF was not associated with a significant difference in IVGTT results between the groups. In steroid-free tacrolimus based immunosuppression the choice of RAPA or MMF did not lead to clinically relevant differences in parameters of glucose metabolism.
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Long-term (4 years) efficacy and safety of pancreas transplantation alone in type 1 diabetic patients

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Background and aims: The role of pancreas transplantion alone (PTA) in type 1 diabetic patients (T1DM) is still debated. This study describes the effects of pancreas transplant alone (single centre experience) on metabolic parameters, cardiovascular risk factors, diabetic complications and kidney function in type 1 diabetic patients followed up to 4 years post-transplant.
Materials and methods: We report our single centre experience on PTA in 71 T1DM (age: 38.4±8.5 yrs; gender: 37 males/34 females; body mass index, BMI: 23.5±3.0 kg/m^2; duration of diabetes: 23.7±9.9 yrs), with a follow-up of up to 4 yrs. Patients were transplanted according to the portal (73.2%) or systemic (26.8%) drainage technique, with enteric diversion of exocrine secretion. Immunosuppression consisted of induction with basiliximab (76.1%) or thymoglobulin (23.9%) and high-dose steroid, followed by mycophenolate mophetil, tacrolimus and low-dose steroid for maintenance.
Results: Patient and pancreas (insulin independence) survival at 4 yrs from transplant were respectively 98.4% and 76.7% with graft losses mainly due to immunological reasons. Relaparatomy was needed in 18.3% of cases, and 15.5% of recipients developed infections. Fasting plasma C-peptide levels rose from 0.15±0.33 ng/ml pre-transplant to 3.00±1.92, 2.78±1.40, 2.36±1.19 and 2.74±1.26 ng/ml at 1, 2, 3 and 4 yrs post-transplant. This was associated with sustained normalization of fasting plasma glucose concentrations and HbA1c levels. In addition, several cardiovascular risk factors (blood pressure, total and LDL-cholesterol and fibrinogen) decreased significantly after PTA and so remained up to 4 yrs of follow-up, with no apparent changes in anti-hypertensive or anti-dyslipidemic pharmacological treatment. Left ventricular ejection fraction, as assessed by ecography, rose from 54.4±4.3 to 57.4±3.2% (p<0.01) . PTA had also beneficial effects on diabetic retinopahy. Pre-transplant 7.5% patients had no retinopathy, and these remained lesion-free at 4-yrs-post operation. Of the 29.5% patients who had non-proliferative retinopathy, 75% improved and 25% remained unchanged, whereas in the gropu with proliferative and/ore laser treated retinopathy, the lesion were stable at 4 yrs post PTA in 82% of subjects and progressed to a more serious grade in the remaining 18%. Protenuria decreased from 1.36±2.72 to 0.29±0.51 g/24h (p<0.01) , which was associated with increased creatinine levels and reduced glomerular filtration rate; however, kidney function changes mainly depended on the pre-transplant status. Finally neuropathy assessment showed a significant improvement of several index of peripheral and autonomic responses after PTA.
Conclusion: We conclude that PTA was effective and reasonably safe in selected type 1 diabetic patients.

PS 25 Differentiation and expansion of beta cells

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***In vivo* GSK3β knockdown hastens endocrine and exocrine pancreatic regeneration in 90 % pancreatectomised rats**

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Background and aims: The Wnt signaling pathway has been recently implicated in pancreas development as well as in beta cell biology. Glycogen synthase kinase 3 β (GSK3β), a pivotal partner of the Wnt signaling is a multifunctional enzyme that negatively regulates the growth and function of the beta cells. The aim of our study was to assess the impact of GSK3β downregulation on the stimulation of exocrine and endocrine regeneration after subtotal pancreatectomy in rat.
Materials and methods: Adult Wistar rats underwent 90% pancreatectomy. In groups of pancreatectomized rats, either antisense oligonucleotides directed against GSK3β (AS-GSK3β) or LiCl were injected directly within the remnant pancreatic tissue immediately after pancreatectomy. Two additional groups were administered with non specific standard oligonucleotides (Std group) or with saline and were used as control for the AS-GSK3β and LiCl treated groups respectively. Beta cell mass was assessed by morphometry 7 days and 4 weeks after pancreatectomy. Beta cell, ductal cells and acinar cell proliferation was measured by BrdU incorporation method 8h and 48h after surgery. Apoptosis was assessed in beta cells, ductal cells and acinar cells by the TUNEL method
Results: GSK3β down regulation via administration of LiCl or AS-GSK3β greatly improved the beta cell regeneration 7 days after surgery (p<0.01). Moreover the use of AS-GSK3β had sustained effect on the beta cell mass, since 4 weeks after surgery the beta cell mass in the remnant pancreas was found to be higher (p<0.05) in AS-GSK3β treated group compared to the Std treated group. The effect of GSK3β inactivation on the beta cell mass seemed to be mediated by the stimulation of beta cell proliferation. Regarding the exocrine pancreas, GSK3β knockdown significantly stimulated acinar cell proliferation. Interestingly, GSK3β inactivation reduced the number of apoptotic acinar cells compared to that found in the control Std group.
Conclusion: Here we show that intra pancreatic knockdown of GSK3β in vivo, promotes both endocrine and exocrine regeneration within the remnant pancreas and could have potential application in the treatment of diabetes and other pancreatic diseases such as pancreatitis.

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Effects of gastrin-treatment on pancreatic metaplasia in pancreatectomised rats

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Background and aim: We have recently found that gastrin treatment improved glucose tolerance and increased beta cell regeneration in pancreatectomized rats. Gastrin stimulated the expression of the crucial transcription factors *ngn3*, *neuroD1*, *pdx-1* and *nkx6.1*, required for beta cell differentiation, in the early phase of regeneration after pancreatectomy (Px). In these initial days pancreatic tissue undergoes remodeling with increased proliferation in the ductal epithelium. The mitogenic activity of the epithelial ductal cells is first seen in the common pancreatic duct and later on in smaller ducts and in metaplastic tissue with tubular complexes (TC), previously identified as "focal areas of regeneration". These regions of regeneration are composed of tubular structures surrounded by mesenchymal cells with high proliferative rate. The aim of this study was to investigate the effects of gastrin on the phenotype of these focal areas of regeneration.
Material and Methods: Sprague-Dawley rats underwent 90%-Px and were treated with [15leu] gastrin-17 (Px+G, n=19) or with vehicle (Px+V; n=20). Pancreatic remnants were harvested on days 1 and 3 after Px and processed for total RNA extraction or paraffin embedding. Gene expression was determined by quantitative real time PCR and protein identification by immunofluorescence.
Results: 3 days after Px, beta cell relative volume was higher in the pancreatic remnants of gastrin-treated rats than in vehicle-treated group (Px+G: 0,57±0,10% vs Px+V: 0,26±0,07%, p=0.03). Beta cell proliferation was similar

in Px+G (2.99±0.32%) and Px+V (3.98±0.35%, *p NS*) groups, suggesting that the higher beta cell relative volume in Px+G group was not due to increased beta cell proliferation. Twenty-four hours after Px, the areas of regeneration accounted for 6.81±1.11% and 5.95±2.16% of the total pancreatic tissue in Px+V and Px+G groups respectively. On day 3, the areas of regeneration had expanded to 38±7.05% and 35.1±5.68% of the total pancreatic tissue (Px+V and Px+G respectively). Two types of TC were identified, TC consisting of many small duct-like cells with strong immunoreactivity for CK20 antibody, and TC formed by few larger cells which exhibit lower levels of immunofluorescence for CK20. TC with small duct-like cells were predominant in Px+V rats, whereas TC with larger cells were more frequent in the gastrin-treated group. Concordantly, gene expression of ductal cell markers (*ck20*, *ca2*, *hnf1β*, *hnf6*) was higher in pancreatic remnants of Px+V rats than Px+G group ($p<0.05$).

Summary and Conclusion: Pancreatic remnants of gastrin-treated rats showed increased beta cell relative volume but similar beta cell proliferation than vehicle-treated rats. In pancreatic remnants from Px+G group, gene expression of ductal cell markers was lower than in Px+V group, and the focal areas of regeneration showed TCs with predominantly larger cells with weak CK20 immunofluorescence. These results suggest that gastrin-induced beta cell regeneration after pancreatectomy may involve phenotypic changes in the metaplastic tissue. Further analysis of these areas of regeneration will provide valuable information to understand the mechanisms of gastrin-induced beta cell regeneration.

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MafA promotes maturation of mouse embryonic progenitor-derived insulin-producing cells

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Background and aims: Shortage of cadervic pancreata has restricted the transplantation therapy of curing insulin-dependent type 1 diabetes. Generation of functional insulin-producing cells from stem cells may circumvent this obstacle. We established a dozen of expansible and glucose-responsive insulin-producing cell lines (e.g. MEPI and ERoSHK) from differentiation of mouse embryonic stem and progenitor cells. Transplantation of these cells in diabetic animals could correct hyperglycemia, but they also revealed features of immaturation as reflected by higher un-regulated insulin release and slow continuous proliferation in vivo. Consistent with the role of MafA (v-maf musculoaponeurotic fibrosarcoma oncogene homolog A) as a master transcription factor implicated in regulating islet β-cell development and glucose responsiveness, its expression was significantly lower in our insulin-producing cells. This study tests the hypothesis that the restoration of MafA levels will improve the function of progenitor-derived insulin-producing cells.

Materials and methods: Mouse embryonic progenitor-derived insulin-producing (MEPI)-1 cells were transfected with lentivirus expressing MafA, followed by assessments of various cell functions. MafA was determined by real time RT-PCR, immunofluorescence staining and immunoblotting.

Results: MafA levels in MEPI-1 cells could be restored to that present in isolated adult islets after 72-h transfection with the virus. Insulin expression and its content were enhanced by about 20% whereas the cell proliferation rate was reduced after restoration of MafA. MafA overexpression significantly enhanced expression of several molecules important for β-cell functions, including glucagon-like peptide-1 (GLP-1) receptor, Glut2, glucokinase, Nkx6.1 and Kir6.2 in MEPI-1 cells. These cells exhibited better signaling responses to glucose stimulation, i.e. higher glucose metabolic rate and ATP generation, enhanced membrane potential depolarization and larger rise of intracellular Ca^{2+} concentrations. Notably, restoration of MafA markedly improved glucose-stimulated insulin secretion (GSIS) profile, revealing a suppressed basal insulin release and shifting the dose-response curve of insulin secretion upon glucose stimulation similarly to that observed in isolated islets. In addition, insulin secretion induced by high K^+, glibenclamide and GLP-1 was also augmented.

Conclusion: Our data demonstrate that normalization of MafA levels in MEPI-1 cells enhances expression of the β-cell relevant gene profile, reduces cell proliferation, elevates insulin biosynthesis and substantially improves GSIS via augmenting multiple signaling cascades. Therefore, we suggest that MafA can promote maturation of embryonic stem cell-derived insulin-producing cells, making them suitable for cell replacement therapy of type 1 diabetes.

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Role of Maf transcription factors in the endocrine cell differentiation in the developing pancreas

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Background and aims: Pancreatic endocrine cell differentiation is dependent on the interactions of several transcription factors. MafA and MafB are essential activators of insulin and glucagon expression. However, MafA and MafB are distinct from others in regards to temporal and islet cell expression pattern, with beta cells only affected by MafB during development and exclusively by MafA in the adult. To elucidate the function of MafA and MafB in β cell differentiation, E18.5 mutant pancreata were analyzed for changes in gene expression. From these gene profiling studies several genes regulated by MafA and MafB factors were identified.

Materials and methods: To identify genes regulated by MafB, expression profiling was performed on wild type and MafB-/- pancreata at embryonic day 18.5. Samples of embryonic and adult tissue were evaluated for candidate genes by qRT-PCR, immunohistochemistry and in situ hybridization.

Results: The microarray studies showed that expression levels of several genes are altered in MafB deficient mice. Gene ontology analysis revealed that the differentially expressed genes were mainly associated with mature beta cell function, such as ion binding and transport, signal transduction, and hormone secretion. This suggests that MafB is involved in beta cell maturation and function. My experiments have shown that Neuronatin (Nnat) and islet-specific zinc transporter (Slc30a8), Endothelin receptor B (Ednrb), Melanocortin 3 receptor (Mc3R), and Microphthalmia associated transcription factor (Mitf) are downregulated in mutant embryonic pancreata. In contrast, the mRNA level of Retinol Binding Protein-4 (Rbp4) was upregulated in mutant tissue. Immunohistochemical analysis shows that Mitf, Ednrb and Mc3r are expressed in the adult pancreas. Endothelin and melanocortin signalling pathways are coupled in the modulation and expression of Mitf gene.

Conclusion: These novel findings indicate a possible involvement of Mitf, Ednrb and Mc3r in the differentiation of pancreatic endocrine cells and adult beta cell function.

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Glucagon-like peptide 1 protein and receptor are present during embryonic life. Biological effects and gene targets

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Background and aims: Glucagon-like peptide 1 (GLP-1) functions during adult life as an incretin hormone with antidiabetogenic properties. However, the role of GLP-1 if any, in early stages of development or in undifferentiated cells, such as human bone marrow mesenchymal stem cells (hMSCs) or mouse embryonic stem cells (mES), remains unknown and it was the aim of this study.

Materials and methods: The presence of GLP-1 and GLP-1 receptor were tested by immunostain of mES and hMSC, as well as in mouse embryos (E 8.5, E10.5 and E13.5). Furthermore, the effects of GLP-1 were tested in hMSC under proliferative, cytoprotective and adipogenic conditions and signalling pathways involved in these processes were also analyzed. Additionally, GLP-1 genes target were studied by quantitative gene expression in a TaqMan Low Density Array.

Results: The isolated hMSCs expressed mRNA and GLP-1 receptor protein. One-day treatment with 10 nM GLP-1 did not modify the stem cell markers SCF, nestin, c-kit and Thy-1, but produced a transcriptional induction of the pancreatic transcription factors neurogenin-3, isl-1 and ipf-1. GLP-1 increased the proliferation of hMSCs, which decreased when they were induced to differentiate into adipocytes. Differentiation produced biochemical and cell morphologic changes with the expression of PPARγ, c/EBPβ, AP2 and LPL in a time-dependent pattern. Interestingly, GLP-1 significantly reduced the expression of PPARγ, c/EBPβ and LPL (inhibition of 30%±4, 33%±2 and 90%±5 respectively). These effects were, at least, exerted through MEK and PKC signalling pathways. In addition, GLP-1 significantly reduced cell apoptosis. In other way, both, peptide and GLP-1receptor were present in mouse embryonic stem cells derived from the inner cell mass where were

found new genes target for GLP-1. Likewise, they were mainly present in cells derived from ectodermal and endodermal linages, in early development of the mouse.
Conclusion: Our data indicate that GLP-1 promotes proliferation and cytoprotection of hMSC, at the same time as it prevents its differentiation into adipocytes, which suggests that this peptide may play a role in the renewal of tissues. Furthermore, both peptide and receptor are present in embryonic life before GLP-1 may play a role as an incretin and modulates the expression of new target genes in mouse embryonic stem cells. These results open new views about the role of this peptide during different periods of life.
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Ablation of FGFR2b in a subset of pdx1+ pancreatic progenitor cells
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Background and aims: Mesenchymal-epithelial interactions mediated by Fibroblast growth factor receptor 2b, FGFR2b, and its ligand FGF10 have been shown to play an important role in early pancreatic development by regulating proliferation and differentiation. However, it is not know if these effects are direct or indirect due to interactions between the pancreatic epithelium and mesenchyme.
Materials and methods: To address the functional role of FGFR2b in pancreatic development in a cell-autonomous fashion, we generated mice with a conditional mosaic deletion of *FGFR2b* under regulation of the *pdx1* promoter. All statistical analyses were performed using GraphPad Prism version 4.03 and t-tests.
Results: This study confirms that *FGFR2b* deletion in a subset of pancreatic progenitors results in a smaller pancreas due to reduced proliferation of pdx1+ cells. We have also successfully narrowed this lack of proliferation down to between embryonic day e13.5 and e14.5. Surprisingly, the cells still retained their ability to differentiate into all lineages of the pancreas, although the ductal development was favored at the expense of the exocrine.
Conclusion: We conclude that proliferation mediated by FGFR2b is cell autonomous while differentiation defects are rescued in the mosaic mouse model, suggesting that there is signaling crosstalk between mutant and wild type cells. Which these secondary signals are and how the differentiated cells behave in terms of migration capacity, maturation status and functionality are still to be determined.
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Gene therapy of diabetic rats by hepatic insulin expression after lentiviral gene transfer
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Background and aims: The release of insulin from non-endocrine cells is an interesting therapeutic concept for the treatment of insulin dependent diabetes mellitus. Modern lentiviral vector systems are able to transduce non-dividing cells and are therefore a valuable tool for gene therapy approaches. The aim of the present study was to normalise the blood glucose concentrations by lentiviral gene transfer of the human insulin gene into hepatocytes of diabetic rats.
Materials and methods: The cDNA of furin-cleavable human insulin was subcloned into a lentiviral vector system. The ubiquitous protease furin allows a processing of proinsulin into mature biological-active insulin in non-endocrine cells. Lentiviruses were isolated by ultrafiltration with a titer of $7x10^9$ infectious particles per ml. Virus solutions were injected into the portal vein of immune-diabetic IDDM and STZ-diabetic rats. Diabetic controls were treated with GFP lentivirus. The blood glucose concentrations and the body weight of the treated animals were measured over one year. 30 and 270 days after virus injection oral glucose tolerance test (OGTT; 2 g/kg body weight) were performed. The serum insulin and c-peptide concentrations were measured by an EILSA. The insulin expression in the liver and the pancreas was analysed by PCR and immunostaining.
Results: 10 days after injection of insulin lentivirus into the portal vein of immune-diabetic IDDM or STZ-diabetic rats the blood glucose concentrations were lowered from 21.6 mmol/l ± 4.6 to 5.9 mmol/l ± 1.1 or from 19.6 mmol/l ± 3.5 to 5.5 mmol/l ± 0.7, respectively. In control rats treated with GFP virus was no significant reduction of the blood glucose concentration detectable. The blood glucose concentration after OGTT of rats injected with insulin virus increased up to 19.7 mmol/l after 30 min and were normalised after 3 h. The serum insulin concentrations increased from <0.1 ng/ml to 2.1 ng/ml after treatment with insulin lentivirus. The rat c-peptide concentrations were below the detection limit. Insulin expression in the liver was detected by PCR and immunostaining. Around 20 % of the hepatocytes were insulin positive. The hepatocytes showed no signs of transdifferentiation into beta cells.
Conclusion: The lentiviral gene transfer of furin-cleavable human insulin into hepatocytes of immune-diabetic IDDM and STZ-diabetic rats leads to a blood glucose normalisation for more than one year. The insulin was processed in the hepatocytes and released constitutively from the cells. This study shows that the diabetic state can be improved by insulin release from non-endocrine cells in a somatic gene therapy approach.

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Modulation of components of Hedgehog signalling pathway in response to beta cell injury
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Background and aims: Pancreatic beta cell mass undergoes major remodeling during neonatal growth and adult life. It has been established that both in Type 1 and Type 2 diabetes there is a significant loss of insulin-producing beta cells and consequently decreased functional beta cell mass. The molecular mechanisms that regulates beta cell mass are still not fully characterized. Since Hedgehog (Hh) pathway plays an important role both in endocrine pancreas development and in the regulation of insulin secretion, we have focused our attention on the contribution of Hh pathway in the regulation of beta cell mass after beta cell injury. To this end, we have studied the expression of Hh signaling key molecules (transcription factors Gli1, Gli2, Gli3) and of Hh receptor Ptch, in C57/BL6 mice in which diabetes was induced by streptozotocin (STZ).
Materials and methods: Six-week old C57/BL6 male mice (n=12) were intraperitoneally injected with a single dose of STZ (200mg/kg b.w.) and then sacrificed and pancreas was collected after 1, 3, 7 and 10 days post STZ administration. In addition, a group of 6-week old C57/BL6 male mice (n=10) not treated with STZ was used as control. The expression of Gli-1, -2, -3, and of the inhibitory receptor Ptch was analyzed on pancreatic sections by indirect immunofluorescence with confocal microscopy analysis. In order to establish the endocrine cell type expressing the Hh molecules of interest, double immunostaining for each of the Hh component and for insulin, glucagon and somatostatin was performed as well.
Results: In untreated mice, Gli1 was expressed both in alpha and beta cells, while Gli2 and Gli3 expression was beta-cell specific. Following STZ administration, Gli1 was not detected in the residual beta cells (1 and 3 days post-STZ) in which it reappeared after 7 days; Gli1 expression remained unchanged in alpha cells after STZ. In contrast, expression of Gli2 and Gli3 did not change in beta cells. Of note, Gli2 and Gli3, which showed a beta-cell specific expression in untreated mice, were detected in alpha cells in a time window between 1 and 3 days post STZ. As for the inhibitory receptor Ptch, this was detected only in somatostatin-positive cells (i.e. delta cells) and its expression was not affected by STZ treatment.
Conclusion: These data show a differential expression, among pancreatic endocrine cell subsets, of Gli transcription factors family members and that such expression is modulated in response to beta cell injury, thus confirming the involvement of Hh pathway in the regulation of beta cell mass. Moreover, the expression of the inhibitory receptor Ptch only in delta cells indicates that in these cells the Hh pathway is inactive and indirectly supports the hypothesis that Hedgehog signaling is indeed involved in the development and regulation of both alpha and beta cells.
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Adaptive response of pancreatic alpha cells and hepatic carbohydrate metabolism during chronic nutritional deprivation

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Background and aims: Type 2 diabetes is mainly characterized by β-cell secretory dysfunction and decreased β-cell mass but inappropriate levels of circulating glucagon and increase in the α-cell/β-cell mass ratio are also important determinants of the hyperglycemia seen in type 2 diabetes patients. In rats, pancreatic β-cell development is particularly sensitive to an altered intrauterine environment what contributes to increase the incidence of adult onset type 2 diabetes. However, little is known about the contribution of α-cells in this context. We previously showed that food-restriction applied during the last third of gestation caused in fetuses at term, an increase of β-cell mass, hyperinsulinemia and enhanced insulin secretion while prolonged malnutrition until adulthood impaired β-cell growth and functionality. Therefore, our first aim was to determine whether maternal food-restriction would affect α-cell growth and functionality in offspring. Since pancreatic hormones play a crucial role in the regulation of hepatic glucose metabolism, our second aim was to examine the adaptive changes of carbohydrate metabolism in response to chronic food deprivation.
Materials and methods: Wistar rats were 65% food-restricted from the last week of gestation until adult age. The experiments were carried out in fetuses at term, in suckling and adult rats. α-cell mass, neogenesis and apoptosis were evaluated by immunocytochemistry and morphometry. For the study of α-cell function fetal and adult islets were isolated. Serum hormone levels, islet glucagon secretion and content were measured by radioimmunoassay. Basal glycogen storage, PEPCK activity and phospho- and total GSK3β protein levels were determined in homogenized livers after fasting.
Results: Undernourished (U) suckling rats were hypoglycaemic. Serum insulin/glucagon ratio was higher in U fetuses and neonates on postnatal day 4 (PN4) as compared to controls (C) but, from PN14 onwards this ratio was lower in U rats. Moreover, glucagon secretion was damaged in U fetuses and adults. α-cell mass increased from fetal period to PN14 then, it remained stable until PN23 and finally, it decreased at adult age in both populations. However, U rats showed defective α-cell mass comparing with C at all the ages studied. The impaired α-cell growth was neither due to increased α-cell apoptosis nor decreased neogenesis but to the presence of islets of smaller size. Liver glycogen content was enhanced in U rats at all the ages studied and this was associated with a higher inhibition of GSK3β. In contrast, PEPCK activity was found impaired from PN14 to adulthood in U rats.
Conclusion: 1) Intra-uterine growth restriction due to maternal malnutrition induces a defective metabolic adaptation of neonates to extrauterine life; 2) The factors involved include morphological and functional alterations of pancreatic α- and β-cells, improved efficiency to store glycogen in the liver but impaired glyconeogenesis; 3) This adaptive process could be crucial to survival during periods of nutritional scarce but detrimental in case of normal diet or overfeeding.
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PS 26 Islet imaging

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Live *in vivo* imaging of Langerhans islets in normal and diabetic mice by extended focus optical coherence microscopy

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Background and aims: Structural and functional imaging of the islets of Langerhans and the insulin-secreting beta cells during diabetes progression represents a significant challenge and a long lasting objective. We have developed extended focus optical coherence microscopy (xfOCM) to image murine islets of Langerhans *in- vivo*. No labeling is required, since the native, intrinsic scattering properties of pancreatic tissues allow for a direct visualization of endocrine islets of various sizes, blood vessels and ductal tree. Moreover, streptozotocin-induced destruction of beta-cells was detected and quantification of this depletion was determined *in-vitro* to evaluate the sensitivity of xfOCM. A longitudinal study performed on NOD mice, a model of type I diabetes, unraveled new perspectives for xfOCM to image onset and progression of diabetes.
Materials and methods: A very same group of NOD mice was repeatedly monitored using xfOCM prior to the development of diabetes, at the early onset of the disease, weeks later and just before sacrifice. Imaging was followed by automated quantification of islet mass. The same animals were regularly sampled for basal glucose measurement tests, intraperitoneal glucose tolerance tests, and body weight measurements. Histological analysis of the pancreata after immunohistochemistry allowed us to characterize the nature of the detected structures.
Results: We show that xfOCM can detect islets of Langerhans in the absence of labeling in mice, and that the islet mass in the sample can be quantified. The longitudinal in vivo imaging study performed on NOD mice shows that (1) we can detect infiltrated islets (2) a decrease in beta cell mass measured by xfOCM is detected prior to detection of impaired glucose tolerance and overt diabetes. The presence of infiltrated islets and beta cell mass disappearance were confirmed by immunohistochemistry.
Conclusion: Our results demonstrate that xfOCM is a suitable tool to assess insulitis and beta cell mass loss prior to impaired glucose homeostasis and diabetes. Therefore, xfOCM will be useful to anticipate a diabetes outcome, since it non-invasively captures islet alterations that cannot be appreciated in vivo by other methods.
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Bioluminescence quantifies alterations of beta cells mass in living Ins-DTR mice

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Background and aims: Studies of β cell loss in the pre-diabetic and diabetic state are impeded by the inability to non-invasively assess pancreatic β cell mass. Non-invasive imaging may also aid the development of therapeutic interventions aimed at mitigating the diabetes-related β cell loss or to regenerating islets. Current methods for quantifying β cell mass and regeneration require the sacrifice of mice and the analysis of pancreatic sections, and do not allow for a sequential evaluation of β cell loss and regeneration in individual mice over time. To assess whether bioluminescence may help to address this problem, we generated MIP-Luc/Ins-DTR double transgenic mice which express the diphtheria toxin receptor (DTR) and luciferase (Luc) under the control of the insulin promoter, i.e. specifically in β cells. In these mice, injection of luciferin permits the emission of photons from β cells, whereas injection of diphtheria toxin (DT) causes a loss of the cells expressing DTR.
Materials and methods: Two-3 month old MIP-Luc/Ins-DTR double transgenic mice, both on a C57BL/6 genetic background, were injected 3 times i.p. with DT at 3 day intervals. When blood glucose was > 450 mg/dl, mice received a s.c. insulin implant. At weekly intervals, bioluminescence imaging (BLI) was recorded from a pancreatic region of interest, 5, 10, 15 and 25 min after the luciferin injection.
Results: In control MIP-Luc mice, the BLI recorded over the pancreas remained stable for the 4 month duration of the experiment. Prior to DT injection, males and females MIP-Luc/Ins-DTR mice had similar values of BLI.

After DT injection, this BLI signal sharply decreased in males (n=5) to about 5-10% its initial value, as the animals developed hyperglycaemia, consistent with a major loss of β-cells. BLI, then, remained at this low level throughout the rest of the experiment. In age-matched females (n=4), DT decreased pancreas BLI by 40-70%, but did not cause hyperglycemia, consistent with the loss of only a fraction of the β-cells. The BLI, then, remained stable for the first 2 months. Thereafter, it increased, suggesting a change in β-cell mass and/or in the efficiency of the photon emission. In this model, the BLI and blood glucose changes differed in males and females, due to the targeted insertion of the DTR transgene to the X chromosome.
Conclusion: The data show that bioluminescence provides for a non-invasive monitoring of graded, and sequential changes in β-cells mass within individual mice.

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Islets transplanted into the anterior chamber of the eye serve as a mirror of *in situ* pancreatic islets

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Background and aims: Since insulin-secreting beta-cells are located in the endocrine pancreas, deeply embedded in the exocrine pancreas, imaging of beta-cells in living organisms is difficult. The anterior chamber of the eye was recently proposed as a transplantation site for non-invasive *in vivo* imaging of pancreatic islets. This location can be used as a natural "body-window" allowing for a repetitive assessment of islet mass and function. The aim of the present study is to demonstrate that islets transplanted into the anterior chamber of the eye are representative of the islets status in the endogenous pancreas.
Materials and methods: We performed syngeneic transplantations into control and obese-hyperglycemic ob/ob mice - a model for type 2 diabetes. Animals were transplanted into the anterior chamber of the eye at 4 weeks of age with islets isolated from age-matched donors. Pancreas and transplanted eyes were collected 3 months later and processed for immunohistochemical studies.
Results: Immunohistochemical staining for insulin showed an altered expression pattern in both ob/ob pancreatic and transplanted islets as compared to control mice. Islets in ob/ob mice showed a strong proliferation rate (1.0 ± 0.2 %) as well as an increased size of beta cells (+30 %), both at the transplantation site and in the endogenous pancreas. The typical increase in intra-islet vessel diameter in the ob/ob mouse was also observed and found to be identical in transplanted islets. We next aimed at demonstrating islet plasticity in the anterior chamber of the eye. Ob/ob mice were treated with daily injections of leptin 2 months after transplantation. After 1 month of treatment, their blood glucose levels normalized and their body weight diminished to reach a similar value as in control mice. Immunostaining at this time point showed that the typical islet properties of ob/ob mice were reversed: quantification of beta cell size, proliferation, and vessel diameters gave similar values as observed in control non-obese mice.
Conclusion: Islets transplanted into the anterior chamber of the eye reflect endogenous pancreatic islets properties. This transplantation site thus serves as a perfect imaging platform allowing for a non-invasive and repetitive assessment of islet function and beta-cell mass regulation at single-cell resolution under normal and diabetic conditions.
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High resolution magnetic resonance imaging detects individual pancreatic islets in whole pancreas

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Background and aims: In type 1 and type 2 diabetes the gradual loss of pancreatic β-cell function and mass leads to impaired regulation of blood glucose with severe secondary peripheral effects. Currently, we lack non invasive methods for monitoring the evolution of the disease. β-cells make-up 80% of the islets of Langerhans which, in the adult, represent 1% of the total pancreatic volume, have a diameter of 30-600 μm and are scattered within the pancreas. Imaging these islets in vivo is the ultimate challenge. To approach this goal, we tested whether the ultra high field of 14.1T MRI, in combination with manganese infusion, can image individual pancreatic islets.
Materials and methods: Mice were untreated (3 mice) or subjected to a MnCl2 i.v. infusion together with a glucose i.p. stimulus (6 mice). MnCl2 and glucose infused mice were further injected i.p. with either streptozotocin (STZ) (4 mice) or citrate buffer (5 mice). In all cases, the pancreata were excised, fixed in 4% PFA for 6h and placed into Fomblin. Samples were then imaged in a 14.1T 26 cm horizontal bore scanner using quadrature half-volume coil 20 mm in diameter. High resolution images were acquired using gradient echo multi slice sequence with fourteen 0.3 mm-thick slices, FOV 26*25 mm, data matrix 512*512 (51*49 μm in plane resolution) to cover the whole mouse pancreas. Two sets of combined T1 and T2* weighted images (TR=282 ms, TE=7 ms, flip=60°, 30 averages and TR=500 ms, TE=14 ms, flip 60°, 20 averages) were acquired for optimal tissue contrast.
Results: The ultra high field of 14.1 T allows for the visualization of different pancreas tissues (lymphatic ganglia, ducts and vessels), whose identity was ascertained by histology. Structures that featured the size, shape and distribution of pancreatic islets were also revealed. The MRI signal of the later structures was enhanced by MnCl2 and glucose infusion, and their islet nature was demonstrated by histological analysis. The distribution of islet areas observed in MRI images was consistent with that evaluated in the histological sections of the very same samples. However, the two distributions were not identical, due to the shrinking of the pancreas during the histological treatment, and to the partial volume effects ascribed to the finite slice thickness of MRI. Preliminary experiments indicate that the approach can detect the loss of islets induced by injection of a diabetogenic dosis of STZ.
Conclusion: We conclude that, when using high resolution MRI together with manganese and glucose infusion, individual pancreatic islets can be visualized within the whole pancreas in the absence of β-cells targeted labeling. The study provides the first proof of concept that a clinically relevant imaging method could be applied for visualizing individual islets in vivo and holds promise for the evaluation of the β-cell changes induced in diabetes.

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Vesicular monoamine transporter 2 gene expression in beta cells of primates but not of rodents: implications for developing radioligands for imaging of beta cell mass in animal models

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Background and aims: Imaging of pancreatic islets and in particular of beta cell mass is a major challenge in diabetes research. Our original observation that the vesicular monoamine transporter 2 (VMAT2) is expressed in the vast majority of human and non-human primate beta cells has led to the suggestion that VMAT2 is a promising biomarker of beta cell mass independent of mechanisms of insulin production. Consequently, radiolabeled analogs of tetrabenazine, a low molecular weight VMAT2 selective ligand, have been employed for pancreatic islet imaging in humans and rats. However, controversial results and limited success in discrimination from background and avoiding overestimation of beta cell mass were obtained. Here we investigated VMAT2 expression patterns in mouse and rat pancreas in order to determine whether these rodents qualify as suitable models to optimize tetrabenazine based beta cell mass imaging.
Materials and methods: Pancreatic tissue was obtained from Wistar, Sprague Dawley and Lewis rats as well as from C57/BL.6 and Balb-C mice. Deparaffinized sections or cryosections from formaldehyde or Bouin Hollande fixed tissues were processed for immunocytochemistry with a panel of species-specific VMAT2 antisera and in situ hybridization in combination with immunostaining for insulin and RT-PCR analysis from laser-microdissected beta cells.
Results: We demonstrated lack of specific VMAT2 expression in beta cells of both mice and rats throughout development. Our antisera revealed an abundant network of VMAT2 positive catecholaminergic innervation costained for tyrosine hydroxylase, the rate limiting enzyme of catecholamine biosynthesis. Absence of VMAT2 transcripts from beta cells was confirmed by RT-PCR analysis from laser-micodissected beta cells. In contrast, beta cells from primates exhibited abundant VMAT2 expression on the mRNA and protein level as previously reported. Thus, it is clearly shown that primate but not rodent species qualify as animal models for beta cell mass imaging with radiolabeled VMAT2 ligands such as 11-C-dihydrotetrabenazine or newly developed VMAT2 biomarkers.

Conclusion: The choice of species is crucial for developing optimal animal models for beta cell mass imaging and requires proof of principle experiments including species-specific gene expression analysis that the biomarker in question is truly valid for both humans and the animal model used. Tetrabenazine remains one of the ligands of choice for imaging of VMAT2 in human pancreas, however existing animal models must be adjusted and/or chosen carefully. Transgenic VMAT2 mice under the insulin promoter may be a way to provide a rodent model for pancreatic beta cell imaging.
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In vivo visualisation of transplanted islets in rat by SPECT imaging with ^{111}In-Exendin-3

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Background and aims: At this moment, there is no reliable non-invasive method to determine beta-cell mass *in vivo*. Such a method would not only allow longitudinal studies on the engrafted islets survival in case of human islet transplantation, but might also enable improved treatment of patients with immune-suppressive drugs to prevent rejection of transplanted islets. We have developed a non-invasive imaging technique that specifically visualizes beta-cells *in vivo*. This method is based on the targeting of the glucagon-like peptide 1 receptor (GLP-1R). The GLP-1R is expressed at high levels on pancreatic beta-cells. We have developed an In-111-labeled tracer that specifically binds to the GLP-1R: In-111-labeled Exendin-3. Previous studies showed a linear correlation between the beta-cell mass and Exendin-3 uptake. We examined whether intramuscularly transplanted beta-cells in rats could be visualized by SPECT (Single Photon Emission Computed Tomography) imaging after i.v. injection with radiolabeled Exendin-3.
Materials and methods: Langerhans islets were isolated from Wag/Rij rats and cultured overnight in RPMI medium prior to transplantation. One thousand islets were transplanted in the left hind limb muscle of Wag/Rij rats, while vehicle was injected in the right muscle as a control (n=3). Two, 7 and 14 days after transplantation ^{111}In-labeled Exendin-3 was injected intravenously and SPECT images were acquired 1 hour post injection using a U-SPECT II microSPECT scanner. After acquiring the SPECT images at the day 14 the rats were euthanized and the radioactivity in the transplant and other relevant tissues was measured. The muscle with engrafted islets was fixed and embedded in paraffin for autoradiography and immunohistochemical analysis.
Results: The transplanted islets were clearly visualized with SPECT at 7 and 14 days after transplantation. *Ex vivo* autoradiography of the engrafted islets showed high uptake of In-111-Exendin-3 in the islets. Immunohistochemistry confirmed that those islets were viable and produced insulin. In-111-Exendin-3 uptake in the muscle with the engrafted islets was higher than uptake in the control muscle (0.27±0.06 %ID vs 0.07±0.02 %ID).
Conclusion: ^{111}In-Exendin-3 accumulated efficiently in transplanted islets. Transplanted islets could be clearly delineated by microSPECT imaging after injection of ^{111}In-Exendin-3. ^{111}In-Exendin-3 could potentially be used for non-invasive beta-cell mass determination of transplanted islets.
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Quantitative determination of the beta cell mass by SPECT imaging with ^{111}In-DTPA-Exendin-3 in rats

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Background and aims: A reliable, non-invasive method that could quantitatively determine the pancreatic beta-cell mass *in vivo* would give further insight in the pathophysiology of type 1 and 2 diabetes. Such a test would allow monitoring effects of diabetes treatments on beta-cell mass, enable individually-tailored therapy and could be used to monitor islet transplantation. The GLP-1 receptor (GLP-1R) is expressed on pancreatic beta-cells. GLP-1, the natural ligand of the GLP-1R, cannot be used for *in vivo* GLP-1R targeting due to its low stability in plasma. Exendin-3 is a more stable analog of GLP-1 and therefore suitable for targeting of the GLP-1R. Exendin-3 was conjugated with DTPA, allowing radiolabelling with ^{111}In. We investigated the potential of SPECT imaging with ^{111}In-DTPA-Exendin-3 to determine the beta-cell mass *in vivo*.
Materials and methods: Diabetes was induced in Brown Norway rats by injecting different doses of alloxan (0, 15, 30, 45, 60 mg/kg) intravenously. Alloxan specifically destroys beta-cells in a dose-dependent manner. One week after alloxan injection, ^{111}In-labeled Exendin-3 was injected intravenously and SPECT images were acquired 1 hour post injection. After SPECT scanning the rats were euthanized and the radioactivity in the pancreas and relevant organs was measured and specific uptake in the Langerhans islets was determined by autoradiography. The beta-cell mass was determined by quantitative analysis of pancreatic sections that were stained immunohistochemically.
Results: In untreated rats, the pancreatic uptake of ^{111}In-DTPA-Exendin-3 was 0.15 ± 0.02 %ID/g. Treatment with 60 mg/kg alloxan resulted in a 80% reduction of pancreatic uptake of ^{111}In-DTPA-Exendin-3 (0.03 ± 0.02 %ID/g). *Ex vivo* autoradiography showed that ^{111}In-DTPA-Exendin-3 specifically accumulated in the endocrine pancreas. The pancreata of untreated rats were clearly visualized on the SPECT images acquired 1 h after injection of ^{111}In-DTPA-Exendin-3. After treatment with 60 mg/kg alloxan the pancreata were non-detectable by SPECT. There was a significant correlation between Exendin uptake and beta-cell mass (r=0.83). The uptake of Exendin as determined by quantitative analysis of the SPECT images correlated with the beta-cell mass (r=0.64).
Conclusion: ^{111}In-DTPA-Exendin-3 can be used to determine the pancreatic beta-cell mass non-invasively by SPECT. The uptake of the tracer is beta-cell specific. The uptake correlated linearly with beta-cell mass and disappeared in diabetic animals without remaining beta-cells. ^{111}In-DTPA-Exendin-3 is a promising tracer for the determination of the beta-cell mass. Clinical trials in healthy volunteers and type 1 diabetic patients are planned.
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Autografting of BMSCs into miniature porcine pancreas for early phase of type 1 diabetes mellitus and tracing it by MRI *in vivo*

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Background and aims: Recently, several researches in rodent animals and T_1DM patients indicated that T_1DM in the early stage might be reversed by autografting BMSCs (ABMSCs). However, the potential mechanisms remained unknown. The aims of the research are to disclose the efficiency and security of autografting BMSCs labeled by super-paramagnetic iron oxide (SPIO) quantitatively into the pancreas of minipig with T_1DM in early stage.
Materials and methods: Nine normal, healthy, 3-month old male Tibetan miniature pigs were randomized into 3 groups, including normal controls (NC), diabetic conventional therapy (DMC) and diabetic autografting BMSCs (DMAB) group. T_1DM pig model were induced by streptozotocin (STZ). T_1DM pigs were administrated with protamine zinc INS via subcutaneous injection to control fasting plasma glucose (FBG) level less than 10.0 Mm/L. In 4-6th wks after successful modeling, DMAB pigs accepted ABMSCs labeled by SPIO into the pancreas under the guidance of digital subtraction angiography. Blood routine biochemical parameters were determined to evaluate the security of ABMSCs at 4 timepoints. At the 3rd and 6th week after ABMSCs, the pancreases of DMAB animals were scanned by 3.0T MR. IVGTT and OGTT were used to evaluate pancreatic beta-cell function and glucose tolerance. Immunohistochemical examination were performed to estimate the regeneration of islets.
Results: After successful modeling, the diabetic animals' FBG were controlled at 5.1-12.5 Mm/L. DMAB group were treated with ABMSCs labeled with SPIO (7.4×10^7,6.8×10^7,7.0×10^7 cells, respectively) through dorsal pancreatic artery. The biochemical parameters of DMAB group had no significant change after ABMSCs. Comparing with pre-ABMSC, the MRI showed scattered low-signal area in the pancreases at the 3rd and 6th wk after ABMSCs. Before ABMSC, the INS dosage and FBG had no significant difference be-

tween DMC and DMAB group. At about 17 to 28 days after ABMSCs, FBG of DMAB group decreased progressively and waved between 2.3-7.5 Mm/L with INS free. IVGTT showed increased INS levels with a delayed peak at 10 min, and improved blood glucose tolerance gradually that had no significant difference comparing with baseline. Pathologic research showed that BMSCs labeled with SPIO differentiated into islet cells or pancreatic ductal epithelial cells in islets which showed positive response in brussian blue staining, and many small neogenetic intact islets in DMAB group and caritive ones in DMC group.
Conclusion: Our data suggested the security and efficiency of quantitative ABMSC through the femoral artery intervention for early stage T_1DM minipigs. ABMSCs could improve islet function effectively and maintain nearly normal FBG free from INS for a period of time. Autografting BMSCs could differentiate into islet cells or improve local microcirculation, and then reverse new-onset T_1DM.
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PS 27 Modulating islets for transplantation

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Rapamycin impairs proliferation of transplanted islet beta cells
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Background and aims: Five years after islet transplantation, only 10 % of patients remain insulin independent revealing a progressive islet dysfunction. The cause of this event is undefined to date but may be the result of adverse effects of immunosuppressive agents. In this study, we examined the effect of rapamycin, a key component of the immunosuppressive regimen in clinical islet transplantation, on islet cell replication *in vivo.*
Materials and methods: Streptozotocin (200 mg/kg i.p.) was used to induce diabetes in NOD/Scid mice at least 5 days before islet transplantation. Five hundred rat islets were transplanted under the left kidney capsule of normoglycemic or diabetic mice. Three days after transplantation, animals were randomly allocated into the experimental groups (control or rapamycin) and BrdU (5 mg/ml) was added to drinking water for 7 days. Mice were treated with rapamycin (0.3mg/kg/every day, i.p.) and control animals received appropriate vehicle treatment. An i.p. glucose tolerance test was performed on all animals at 10 days. Beta cell replication was determined by double immunofluorescence staining for insulin and BrdU. Data are expressed as % positive BrdU beta cells and as mean ± SEM for 3 or more independent experiments.
Results: Although control and rapamycin-treated mice had similar blood glucose levels before and 2h after i.p. glucose injection, both the peak glucose levels and duration of the glucose excursion (AUC in mM of glucose over 120 min) were increased in rapamycin-treated mice when compared to control mice (830±95 vs. 284±42; rapamycin vs. control, P=0.0004). In non streptozotocin-treated mice, rapamycin decreased the % of BrdU positive beta cells within endogenous islets (0.66±0.16 vs. 1.71±0.41; rapamycin vs. control, P=0.029). Furthermore, rapamycin reduced the % of BrdU positive beta cell in transplanted islets (0.71±0.16 vs. 4.60±0.42; rapamycin vs. control, P<0.0001). Similar results were obtained with Ki67 staining as an alternative mean of identifying proliferating cells (0.08±0.04 vs. 0.80±0.17; rapamycin vs. control, P=0.002). When streptozotocin-treated mice were treated with rapamycin, the % of BrdU positive beta cell in the graft was also significantly decreased (1.39±0.26 vs. 3.96±0.71; rapamycin vs. control, P=0.004). Finally, the apoptotic rate in transplanted islets was very low and no significant difference was observed between control and rapamycin-treated mice.
Conclusion: Our results indicate that rapamycin reduces the rate of pancreatic beta cell proliferation in transplanted rat islets but also in native pancreatic murine islets. It is therefore suggested that progressive graft islet dysfunction may result in part from an impairment of beta cell regeneration induced by rapamycin in transplanted patients.
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Differences in blood perfusion correlates to pancreatic islet function *in vitro*
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Background and aims: The blood perfusion between different pancreatic islets varies considerably. In recent experiments we observe that this result in a markedly heterogenous oxygenation of the islets. The present study tested the hypothesis that heterogeneity between islets with regard to vascular support is also reflected in differences in islet beta-cell function.
Materials and methods: Fluorescent microspheres (10 µm in size) were used to measure the blood perfusion of individual pancreatic islets in adult Wistar-Furth rats. Based on the microsphere distribution islets were separated into two groups, with blood flow below or above 0,4 µl/min. Functional studies of glucose-stimulated insulin release, insulin content and glucose oxidation rate were performed *in vitro* on freshly isolated islets. Gene expression studies were performed by RT-PCR. Vascular density quantification using two-photon confocal microscopy was performed separately for each group of islets after intravascular visualization of blood vessels by IB_4 isolectin.

Results: Functional studies on freshly isolated islets of the two groups revealed that islets with better blood perfusion had much higher basal and glucose-stimulated insulin release when compared to less perfused islets. No differences were observed between groups with regard to total insulin content, mitochondrial function, as assessed by studies of glucose oxidation rate, or in islet size. Vascular density quantification showed that approximately 10% of islets were composed of blood vessels in both groups, but that the vasculature in islets with lower blood perfusion had less tortuous architecture. Moreover, preliminary studies suggest that islets with better blood perfusion have higher gene expression of glucose transporter 2.
Conclusion: Our results indicate that pancreatic islets with higher blood perfusion also have better function that remains after isolation of the islets. This may partially be explained by differences in vascular structure, but need to be further investigated.
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Hypoxia-protective effects of preconditioning in beta cells are exerted at the level of insulin biosynthesis and are mediated by inhibition of calcium inflow
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Background and aims: Pharmacological preconditioning alleviates the impact of hypoxia in heart and brain; therefore similar procedures may also adapt beta cells to hypoxia prior to transplantation and this could be therapeutically useful. We have found that preconditioning in vitro by the K-ATP-channel opener diazoxide alleviates the diminution of cellular insulin contents brought about by experimental hypoxia on rat pancreatic islets. Here we report on possible mechanisms behind these beneficial effects.
Materials and methods: Rat or human islets were maintained in tissue culture (RPMI, 11mM glucose). They were subjected to 5.5 h of hypoxia with or without a 22h period of preconditioning with diazoxide and/or other agents.
Results: Rat islet insulin contents were reduced by 23 % after hypoxia and by 61% after a further 22 h re-oxygenation period. Preconditioning with diazoxide (325 µmol/l) alleviated the hypoxia effect (2.7 fold increase, $p < 0.001$ vs. hypoxia alone). Hypoxia reduced proinsulin biosynthesis (^{3}H-leucine incorporation into proinsulin) by 35 ± 6 % and this decrease was partially corrected by preconditioning (by 91 %, $p < 0.03$). Beneficial effects of diazoxide were abolished by including tolbutamide or elevated potassium (i.e. conditions which increase calcium inflow) during preconditioning. Preconditioning with 10 µmol/l of nifedipine, a calcium channel blocker, reproduced the beneficial effects of diazoxide. We employed cooling, i.e. culture at 28 C before hypoxia, to dissociate effects on insulin secretion (inhibited by cooling) from effects on calcium inflow (marginally affected by cooling). Cooling inhibited glucose induced insulin secretion by 64 ± 2 % but did not induce any positive preconditioning effect, instead a negative one was recorded (-40 ± 6 %). Both diazoxide and nifedipine moderately but significantly inhibited glucose oxidation before the period of hypoxia.
Conclusion: 1) hypoxia-induced functional deficits are alleviated by prior blocking of calcium inflow, probably acting through attendant effects on mitochondrial metabolism, 2) both agents which block calcium inflow indirectly and those which block directly have therapeutic potentials.
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Composite pig islet-human endothelial progenitor cell grafts can reduce the instant blood-mediated inflammatory reaction
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Background and aims: One of obstacles in clinical islet transplantation is the islet loss in the early post-transplant period, which is as much as 50-60% of the grafts. This mainly results from intravascular islet-induced nonspecific inflammatory and coagulation pathways promoting a so-called instant blood-mediated inflammatory reaction (IBMIR). Endothelial cells are known to protect against complement-mediated lysis and activation of coagulation. Endothelial progenitor cells (EPC) seem to have lower profile of procoagulation in some environment compared to mature endothelial cells. EPC can be isolated from peripheral blood, and be expanded ex vivo, which is important as for clinical application. In addition, EPC display a unique ability to promote angiogenesis although the underlying molecular mechanism remains poorly understood. These suggest that EPC might be a better source to protect IBMIR. Therefore, we tested effects of composite pig islet- human EPC grafts in vivo.
Materials and methods: Porcine islets were cocultured with human EPC overnight to obtain about 50% coverage. The cells were transplanted to STZ-diabetic athymic (nu/nu) nude mice through portal vein. Daily body weight and fed blood glucose were monitored, and serial harvest of liver tissues was performed for morphologic analysis.
Results: Composite pig islet-human endothelial progenitor cell grafts significantly improved blood glucose levels compared to islet grafts immediately after transplantation and then for 1 week, which suggested better graft survival from IBMIR. On morphologic examination, transplanted composite grafts showed a reduction in leukocyte infiltration and coagulation, and stained positive for insulin and human lectin demonstrating presence of both islets and EPC.
Conclusion: If optimal EPC-islet coculture method can be identified, EPC-coating has potential as a candidate that can control the strong innate immune response induced by islet grafts transplanted through the portal vein, and clinical islet transplantation would be more available and feasible strategy for the cure of diabetes.
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Co-transplantation with mesenchymal stem cells improves islet transplantation outcome in mice
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Background and aims: Islet transplantation is a potential cure for Type 1 diabetes, but despite recent advances most patients revert to hyperglycaemia within five years. It is thought lack of success may be partially due to beta cell death in the immediate post transplantation period and poor revascularisation. Mesenchymal stem cells (MSCs) are adult progenitor cells which play a major role in tissue repair. The aim of the study was to investigate whether co-transplantation of MSCs with islets could improve transplantation outcomes in mice.
Materials and methods: Mesenchymal stem cells were isolated from the kidneys of C57Bl/6 mice and were expanded *in vitro*. Islets were isolated from the pancreases of donor C57Bl/6 mice. Streptozotocin-diabetic C57Bl/6 mice were transplanted with a suboptimal graft of either 150 islets alone or 150 islets + 250,000 MSCs under the kidney capsule. Blood glucose concentrations were monitored for 28 days after which the graft-bearing kidneys of cured mice were nephrectomised. The islet grafts and endogenous pancreases were analysed histologically for insulin, glucagon and CD34 as markers for beta, alpha and endothelial cells, respectively.
Results: Prior to transplantation, blood glucose concentrations were 27.6 ± 1.8 mM and 28.2 ± 1.3 mM in the islet alone and islets + MSC groups, respectively. After transplantation, the mice that were implanted with islets + MSCs had lower blood glucose concentrations than mice implanted with islets alone (day 7: 11.6 ± 2.2 mM vs 27.5 ± 2.1 mM, day 14: 11.1 ± 2.1 mM vs 26.9 ± 2.0 mM, day 28: 8.6 ± 1.0 mM vs 19.0 ± 3.6 mM, n=9, $p<0.01$, 2 way RM ANOVA with Bonferroni Post-Hoc test). After 28 days, in cured mice (non-fasting blood glucose<11.1 mM: 8 of 9 mice in islets + MSC group, 3 of 9 mice in islet alone group) the graft bearing kidney was removed, after which all mice reverted to hyperglycaemia (>20 mM). The density of endothelial cells was increased in the islet + MSC grafts (978 ± 92 endothelial cells/mm^2) compared to the islet alone grafts (702 ± 23 endothelial cells/mm^2, n=4-6, $p<0.01$, t-test). No differences were seen in the endogenous pancreases between the two groups with regard to size of islets and frequency of insulin and glucagon staining, with most cells in the islet remnants consisting of alpha cells.
Conclusion: Co-transplantation with MSCs improves syngeneic islet transplantation outcome in mice. This is associated with increased numbers of endothelial cells in the graft and altered islet graft morphology. There is no difference in insulin positivity in the endogenous pancreases between the groups, indicating that the positive effects of MSCs are indeed a direct effect on the graft.
Supported by: RCUK

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Gene transfer of glucagon-like peptide-1 to mouse islets improves transplantation outcome

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Background and aims: Pancreatic islet transplantation (TPL) is a promising therapeutic intervention for T1DM. One of the significant obstacles to successful islet TPL is a high number of cell deaths in islet grafts during the early days after TPL, mostly from inflammatory and hypoxic damage. To overcome these obstacles and improve the outcome of islet TPL, the transfer of cytoprotective genes to isolated islets has been attempted. Because glucagon-like peptide-1 (GLP-1) was shown to stimulate beta-cell proliferation and have anti-apoptotic effects on beta cells, we examined whether adenovirus-mediated gene transfer of GLP-1 would result in cytoprotection of islets in vitro and in vivo TPL setting.
Materials and methods: Isolated mouse islets were transduced with an adenoviral vector coding for GLP-1 (rAd-GLP-1) or green fluorescent protein/beta-galactosidase (rAd-GFP/LacZ). After transfection, GLP-1 expression was assessed by RT-PCR of islet mRNA and RIA of culture media. To assess the in vitro cytoprotective effect, transduced islets were treated with H2O2 (200 microM for 30 min) and cell death and MMP were measured using AO/PI and JC-1. To assess the effect of GLP-1 expression on islet survival in vivo, suboptimal mass of transduced islets was transplanted into renal subcapsular space of syngeneic diabetic mice.
Results: Adenoviral delivery of GLP-1 to islets resulted in dose (m.o.i.)-dependent increase in synthesis and secretion of GLP-1. Islets transduced with rAd-GLP-1 were protected from H2O2-induced cell damage in vitro. Two weeks after TPL, diabetes cure rate with islets transduced with rAd-GLP-1 (81%) was significantly higher than that with islets transduced with rAd-GFP/LacZ (25%).
Conclusion: Theses results indicate that the overexpression of GLP-1 in islets enhances islet survival in vitro and preserves islet function in transplants. Our results suggest that GLP-1 expression in islets is one of plausible and useful strategies for ex vivo cytoprotective gene therapy in islet TPL.
Supported by: IRICT, Republic of Korea

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The thyroid hormone T3 improves function and survival of rat pancreatic islets during *in vitro* culture

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Background and aims: Islet cell transplantation is an alternative therapy to conventional ones for type 1 diabetic patients. However, this strategy is severely limited by the shortage of organ donors. Ex vivo islet cell culture prior to transplantation is a good alternative, but the maintenance of islets in culture is at the moment a difficult task. Therefore, stimulation of islet proliferation and differentiation in vitro remains the major scientific and clinical goal. Many growth factors have been studied, and related to intracellular signalling molecules and cell cycle regulators playing key roles in pancreatic beta cells. Among these, the kinase Akt has been indicated as a crucial intracellular mediator of beta cell growth and survival both in vitro and in vivo studies. We previously demonstrated that thyroid hormone T3 can increase beta cell function via specific activation of Akt, therefore we suggest that the addition of T3 to primary rat islets culture could preserve islets features from their physiological degradation, improving their status prior to transplantation.
Materials and methods: Rat pancreatic islets, isolated by collagenase digestion, have been cultured in the presence or not of T3 10-7M. Immunofluorescence analysis has been performed to evaluate the expression of thyroid receptor beta1 in rat islets. The islets viability has been studied by the use of two different dyes, one cell permeable green fluorescent dye and propidium iodide, and by the analysis of core cell damage upcoming. Beta cell proliferation within islets was analyzed by BrdU incorporation. Moreover, islet function has been evaluated by measuring insulin secretion. The ability of beta cells to counteract apoptosis induced by streptozotocin has been analyzed by TUNEL assay, and the Akt activation by Western blotting analysis.
Results: We demonstrated that treatment of primary cultures of rat pancreatic islets with T3 results in increased beta cell vitality with an augment of their functional properties. Contemporary a sensible reduction of the core damage has been observed in T3 treated islets, showing a preservation of the beta cells integrity during the culture period. Furthermore, when BrdU incorporation was performed, a much higher number of positive nuclei in T3 treated islets indicates that T3 not only improves islet status, but also that induces beta cell proliferation. Nonetheless, the insulin secretion is sensibly augmented after T3 stimulation. All the observed effects were associated with a strong increment in Akt activation, suggesting the involvement of this kinase in T3-mediated phenomena in pancreatic islets.
Conclusion: Our observations indicate the thyroid hormone T3 as a suitable factor to optimize and stimulate recovery and subsequent function of islets during in vitro culture suggesting that thyroid hormone could play an important role in physiological function of pancreatic islets.
Supported by: IRICT, Republic of Korea

PS 28 Mitochondria in beta cells

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Transcriptome analysis of type 2 diabetic islets: evidence of mitochondrial dysfunction

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Background and aims: Type 2 diabetes (T2D) is a multifactoral syndrome, with genetic and environmental factors causing a progressive beta cell dysfunction. The molecular alterations affecting T2D islets are not fully understood.
Materials and methods: We performed microarray analysis, followed by quantitative PCR of a few selected genes, of isolated islets from 6 T2D (age: 71±9 yrs; gender: 3M/3F; BMI: 26.0±2.2 Kg/m2) and 7 non-diabetic (ND, age: 58±17 yrs; gender: 4M/3F; BMI: 24.8±2.5 Kg/m2) subjects. RNA was hybridized on Affymetrix chips (HG U133A). After quality control by *affy* and *affyPLM*, gene expression intensity values were normalized by Robust Multi-array Average (RMA), whereas differential expression was assessed by *limma*. Functional studies were also performed with isolated islets.
Results: When T2D islets were compared to ND, the expression of 1345 probe sets resulted ($p<0.01$ and fold change of <0.5 and >2.0) different; of these, 59 were up-regulated and 1286 down-regulated. Overall, they identified 1230 genes, related to several beta-cell features. By using Gene Ontology and KEGG databases, we observed that those genes influenced 21 processes and 13 pathways, respectively. All the differences were confirmed by Gene Set Enrichment Analysis, which showed that, of the 1419 gene sets analyzed, 195 were positively and 42 negatively enriched in T2D samples. In all the analyses a reduction of the expression for genes involved in oxidative phosphorylation and citric acid cycle was observed. Accordingly, by qPCR a significant ($p<0.02$) decrease (-42%) of succinate dehydrogenase (SDH) subunit B expression was detected. When ND islets were exposed to methyl malonic acid, an inhibitor of SDH activity, a reduction (-33%) of glucose-stimulated insulin release was observed, that was accompanied by an alteration of the ADP/ATP ratio.
Conclusions: In conclusion, type 2 diabetic islets show many alterations of transcriptome, including changes of genes involved in mitochondrial ATP production; it remains to understand which alterations are cause or consequence of the diabetic condition.
Supported by: EURODIA

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Mitochondrial proteome analysis reveals changes in expression of multiple proteins in pancreatic beta cells exposed to high glucose

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Aims: Chronic hyperglycemia leads to deterioration of insulin release from pancreatic β-cells as well as insulin action on peripheral tissues. However, the mechanism underlying β-cell dysfunction resulting from glucose toxicity has not been fully elucidated. The aim of present study was to define a set of alterations in mitochondrial profiles of pancreatic β-cell lines using two-dimension gel electrophoresis (2-DGE) and mass spectrometry.
Material and methods: INS1E cells were incubated in the presence of 5.5 and 20 mM glucose for 72 hrs. An aligate of media was removed for measurement of insulin release (RIA) and the cells were subjected then either for isolation of mitochondria or were frozen for western blot analysis , protein profile determined by two-dimension gel electrophoresis (2-DGE) and mass spectrometry.
Results: More than 400 spots were detected on the colloidal coomassie stained 2-D gels; of these protein spots, 75 displayed two fold or more significant change ($p>0.05$) in relative abundance in the presence of 20mM glucose compared to the control. Thirty-three protein spots appear only on the control mitochondrial map. Mitochondrial proteins down regulated in glucotoxic conditions includes ATPsynthase α chain and δ chain, malate dehydrogenase, aconitase, trifunctional enzyme β subunit, NADH-cytochrome b5 reductase and VDAC2.There was up regulation of VDAC1, GPR75, HSP60 and HSP10. Protein identification revealed contamination of the mitochondrial fraction with proteins from other organelles. These differentially expressed proteins includes proinsulin, calreticulin, PD1A6, PKCsubstrate60.1kDa protein ORP150, endopalsmin, HSC70, heterogeneous nuclear rib nucleoproteins D0 and A2/B1, lamin B1, histones H2B, H3.3 and H4 and elongation factor 1-α-1. In conclusion, orchestrated changes in expression of VDAC and multiple proteins involved in nutrient metabolism, ATP synthesis, cellular defence, glycoprotein folding, apoptosis signaling and mDNA stability may explain cellular dysfunction in glucotoxicity resulting in altered insulin secretion.
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Novel islet respirometry assay reveals high levels of uncoupled respiration in rodent and human islets

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Background and aims: Mitochondrial metabolism is essential for proper insulin secretion as oxidative phosphorylation produces the majority of the cells ATP required for insulin granule exocytosis. However, mitochondrial substrate oxidation is not fully coupled to ATP synthesis as part of the proton gradient across the inner mitochondrial membrane reenters the matrix through other ways than ATPsynthase; this is termed uncoupled respiration or proton leak. In this study we sought to characterize the basal proton leak of intact islets, it's regulation as well as its profile in diseased islets.
Materials and methods: To enable this study we developed a high-throughput islet respirometry approach based on the XF24 platform, originally designed to study monolayers of cells. By applying drugs that act on the respiratory chain we can estimate the level of fuel-stimulated, uncoupled, maximal as well as non-mitochondrial respiration under various conditions. Islets were derived from wildtype and high fat diet fed C57Bl6/J mice as well as from human donors.
Results: When stimulated with fuels mouse islets displayed a marked increase in respiration. The basal level of islet uncoupled respiration was measured to 55%, strikingly higher than other cell types. We found that cellular fuels such as amino acids, free fatty acids and glucose significantly uncouple islet mitochondria and that this is prevented by antioxidants. Further we found that the adenine nucleotide transporter, but not the permeability transition pore, makes a significant contribution to the proton leak. In vitro incubation with palmitate did not affect the level of uncoupled respiration under low glucose, but had a significant effect when the islets were stimulated with high glucose. Further, islets from high-fat diet-fed mice exhibited higher levels of uncoupled respiration compared to islets from chow fed control animals. Islets with beta-cell specific deletion of uncoupling protein 2 (UCP2) were found to exhibit normal levels of uncoupled respiration pointing to UCP2 not being a major contributor to the measured uncoupled respiration. In addition to the studies on mouse islets, human islets from healthy as well as diabetic donors were tested. Human islets also display high levels of uncoupled respiration that is increased by high glucose.
Conclusion: Islets have relative high levels of uncoupled respiration, which is regulated by cellular fuels and reactive oxygen species. Adenine nucleotide transporter but not UCP2 or permeability transition pore appears to contribute to the observed uncoupled respiration. Interestingly levels of uncoupled respiration increase in a diabetes animal model. In principle, tuning islet mitochondrial efficiency may represent a therapeutic target.
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Oxidative phosphorylation-dependent insulin secretion in pancreatic beta cells: new insights by disrupting TFB1M

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Background and aims: Oxidative phosphorylation (OXPHOS) is a mitochondrial metabolic pathway that uses energy released by the oxidation of nutrients to produce ATP. It depends on the functional status of the mitochondrial electron transport system (ETS). Disrupting the mitochondrial transcription factor B1 (TFB1M) impairs the function of the ETS complexes

as TFB1M regulates the translation of mitochondria encoded subunits. The fact that these subunits are of different importance for the individual ETS complexes allows us to investigate the metabolic regulation of the ETS activity, OXPHOS, and therefore insulin secretion in glucose-responsive INS-1 832/13 clonal beta-cells in detail.
Materials and methods: Knock down (KD) of TFB1M was achieved by RNA interference. Oxygen consumption rates were measured using the Seahorse Extracellular Flux Analyzer XF24. Stable isotope-labeling with amino acids in cell culture (SILAC) and immunoprecipitation were used for proteomic analysis of the ETS complexes. ATP levels were detected by luciferase assay. Insulin secretion was determined by RIA.
Results: SILAC analysis and detection of TFB1M by immunoblotting confirmed its knock down by more than 50% in INS-1 cells. In intact cells, as predicted, TFB1M knock down significantly decreased the glucose-stimulated ADP-activated coupled OXPHOS capacity P (10.9 +/- 0.3 nmol O2/min/mg protein vs. 9.0 +/- 0.5 nmol O2/min/mg protein; P<0.01) and electron transport capacity E of the ETS (15.5 +/- 0.5 nmol O2/min/mg protein vs. 12.1 +/- 0.9 nmol O2/min/mg protein; P<0.05) as well as mitochondrial ATP generation and triggering pathway-dependent insulin secretion. Immunoblotting and SILAC analysis showed a decreased expression level of nucleus and mitochondria encoded ETS subunits, foremost of complex I, which also showed the most extensive decrease in activity to 40.7 +/- 2.8 % of the activity in control cells. Consistently, measurements with defined metabolic substrates supporting ETS complex I activity in permeabilized cells demonstrated that P (47.9 +/- 0.8 nmol O2/min/mg protein in control vs. 33.9 +/- 1.3 nmol O2/min/mg protein in TFB1M KD; P<0.01) and E (75.3 +/- 5.9 nmol O2/min/mg protein in control vs. 50.4 +/- 3.5 nmol O2/min/mg protein in TFB1M KD; P<0.01) were affected significantly. However, measurements with substrates employing complex II showed no significant differences in P and E, despite pronounced TFB1M knock down-dependent decreases in complex III and IV activity to 60.1 +/- 4.7 % and 74.5 +/- 3.5 % of the activity in control cells, respectively.
Conclusion: (1) ETS complex I activity is rate-limiting for the metabolite-stimulated electron transport of the ETS and therefore OXPHOS and ATP generation in beta-cells. (2) 832/13 clonal beta-cells employ significant excess capacity of complex III and IV activity (3) Mitochondrial OXPHOS is crucial for the fuel stimulation of insulin secretion. (4) In INS-1 832/13 clonal beta-cells, disruption of mitochondrial translation profoundly affects mitochondrial function, predominantly by diminished complex I stability. Thus, based on these novel findings, further studies concerning the mechanisms of metabolic regulation of ETS activity and OXPHOS and therefore mitochondria-based metabolic coupling of insulin secretion open a new avenue for research in pancreatic islet cell biology.
Supported by: VR

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The Atp8 mutation of complex V impairs secretory function of beta cells - role of mitochondrial ROS and ATP in response to glucose challenge
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Background and aims: The conplastic mouse strain B6mt^{FVB} carries a stable mtDNA mutation of the *Atp8* gene affecting the assembly of the Fo subunit of the ATP-synthase complex. It was the aim of the study to correlate secretory responsiveness to glucose with ATP/ADP levels and mitochondiral ROS generation in comparison to the B6mt^{AKR} control strain.
Materials and methods: B6mt^{FVB} and B6mt^{AKR} were regularly monitored for blood glucose and tested for glucose tolerance feeding standard diet. Pancreatic islets were isolated from normoglycaemic mice (age 12 - 16 weeks) and cultured at 5 or 30 mmol/l glucose for 24 h and 48 h. Production of mitochondrial superoxides was measured by the Mitosox^{TM} indicator using fluorescence microscopy. Cellular ATP and ADP levels in isolated islets were measured by a luminometric assay. Glucose stimulated insulin secretion of isolated islets was measured by ELISA.
Results: B6mt^{FVB} as well as B6mt^{AKR} mice showed normal blood glucose levels (5.8 ± 0.4 vs. 6.8 ± 0.6 mmol/l). Both strains showed a normal glucose tolerance when challenged with 1 g glucose/kg b. wt. Exposure of isolated B6mt^{FVB} islets to 30 mmol/l glucose for 24 h or 48 h resulted in a significant decrease of glucose-stimulated insulin secretion by 80 % ($p < 0.001$) and 50 % ($p < 0.05$) in comparison to B6mt^{AKR} islets. Basal as well as glucose-stimulated (2.8 vs. 20 mmol/l) insulin secretion were not different between the two mouse strains when the islets were pre-cultured at 5 mmol/l glucose for 24 h or 48 h. Islets from B6mt^{AKR} mice showed a 4-fold increase of ATP levels when glucose concentrations were increased from 2.8 to 20 mmol/l. ATP levels in islets from B6mt^{FVB} mice were insensitive to changes of glucose. The ATP/ADP ratio was 2.5-fold higher in islets from B6mt^{AKR} mice in comparison to the B6mt^{FVB} strain after 24 h preculture at 30 mmol/l glucose. In islets from B6mt^{FVB} mice mitochondrial ROS generation increased significantly at 30 mmol/l glucose while there were only slight increases in islets from B6mt^{AKR} mice. However, mitochondrial ROS generation at 5 mmol/l glucose was higher in islet cells from B6mt^{AKR} mice than in islets from the B6mt^{FVB} strain. Preculture of islet cells at 30 mmol/l glucose for 24 h or 48 h did not significantly affect cell viability in the B6mt^{FVB} and B6mt^{AKR} strain.
Conclusion: The beta cell dysfunction in islets with the *Atp8* gene mutation is plausibly explained by reduced ATP production through complex V of the respiratory chain. In line with metabolic stimulus-secretion coupling this resulted in reduced glucose-responsiveness of insulin secretion. Mitochondrial ROS generation in B6mt^{FVB} islets is more sensitive to high glucose than in B6mt^{AKR} islets. Our data provide evidence that in islets with *Atp8* gene mutations adaptive changes of mitochondrial ROS generation rather than absolute levels are important to induce beta cell dysfunction under conditions of nutrient stress.
Supported by: German Diabetes Association (DDG)

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Marked, but not moderate induction of uncoupling protein 2 enhances selected aspects of beta cell mitochondrial metabolism and decreases susceptibility to toxicity
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Background and aims: The effects of uncoupling protein 2 (UCP-2) for mitochondrial metabolism, for beta cell function and for propensity of diabetes have been intensely studied. However, because of conflicting results no agreement on the role(s) of UCP-2 has been reached. Different degrees of induction or inhibition of UCP-2 between studies could be an important but unexplored reason for conflicting results. Here we investigated effects of a fourfold induction on mitochondrial, insulin and toxicity parameters and tested for replication of positive findings at a lower level of induction.
Materials and methods: We transfected INS-1 cells to obtain a tet-on inducible cell line. A 48 h exposure to 1 μg/ml of dox induced UCP approximately fourfold (424 ± 113 %, mean ± SEM) and 0.1 μg/ml twofold (178 ± 29%, n = 3).
Results: Fourfold induced cells displayed normal mitochondrial membrane potential (by Rhodamine 123), normal mitochondrial mass (Mitotracker Green) and normal ATP levels. By immunoblotting subunits of complex 1 (ND6) and 2 (FeS) and 4 (COX I) were not changed. However, core2 for complex III was up-regulated 17 %, and α subunit for complex V by 20%. Glucose oxidation (at 11 mM glucose) was not significantly affected (+5 ± 9 %, n = 6), however, oxidation of fatty acids (^{14}C-oleate) was increased by 35 ± 15 % ($p < 0.05$, n = 12). Cellular insulin contents (-4 ± 7 %, n = 3) and glucose-induced insulin secretion were not affected (+27 ± 11 %, n = 5), nor insulin responses to oleate. A fourfold induction protected against H_2O_2-induced toxicity by 22 ± 5 % ($p < 0,01$, n = 8, MTT assay and flow cytometry). However, the lower (approximately two-fold) induction of UCP-2 did not reproduce mitochondrial and metabolic effects, nor protection against toxicity effects (and susceptibility towards the latter was not aggravated by UCP-2 down-regulation by siRNA).
Conclusion: A fourfold induction of UCP-2 induces subtle but definite enhancing effects on mitochondrial metabolism and also toxicity protection. However, lack of reproducibility at lower levels of induction as well as lack of a negative effect on insulin parameters argues against an important role of UCP-2 in beta cells in diabetes.

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Cytosolic phosphate, a regulator of mitochondrial function in insulin-secreting cells

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Background and aims: Mitochondrial energy metabolism depends on the continued uptake of ADP and inorganic phosphate as substrates of the ATP synthase and the export of the product ATP from the organelle. The exchange of ATP against ADP is mediated by the adenine nucleotide translocase (ANT). Uptake of inorganic phosphate by mitochondria is linked to the net uptake of one proton per phosphate anion. Here we tested whether inorganic phosphate regulates mitochondrial energy metabolism in INS-1E cells in addition to its role as a substrate for the ATP synthase.
Materials and methods: Mitochondrial ATP synthesis was studied in staphylococcal alpha-hemolytic toxin permeabilized INS-1E cells. This treatment leaves the membranes of intracellular organelles such as mitochondria intact and allows us to distinguish between intraorganellar ATP and the ATP pool released from mitochondria. ATP was measured with a bioluminescence assay. A fluorescence microscopic imaging system was used to study the mitochondrial matrix pH and a microplate reader to record the mitochondrial membrane potential with JC-1.
Results: Permeabilised INS-1E cells showed mitochondrial membrane hyperpolarisation and matrix alkalinization in response to substrates such as succinate or glycerolphosphate. We find that the hyperpolarisation was strongly dependent on the extramitochondrial phosphate concentration. Phosphate addition alone induced mitochondrial matrix acidification in permeabilised cells. Matrix alkalinization induced by succinate was less dependent on the extramitochondrial phosphate concentration than mitochondrial hyperpolarisation. ATP release from mitochondria induced by substrate but not the increase of the organellar pool of ATP was completely blocked by atractyloside, an inhibitor of the ANT. ATP release was also strictly dependent on the presence of extramitochondrial ADP, implying that ATP release from mitochondria is exclusively dependent on ANT activity. Upon stimulation with mitochondrial substrates, the amount of ATP exported from mitochondria was markedly accelerated by increased phosphate concentrations contrasting with the modest elevation of ATP inside the mitochondria.
Conclusion: We demonstrate that the cytosolic phosphate concentration has a strong impact on the mitochondrial electrochemical gradient, ATP synthesis and ATP export from the organelle. Our results suggest that inorganic phosphate affects mitochondrial energy metabolism beyond its role as a substrate for ATP synthesis. As large changes in phosphate transport across the plasma membrane have been reported in islets, our results emphasize the importance of cytosolic inorganic phosphate concentrations in metabolism-secretion coupling.

PS 29 Glucose and mitochondrial metabolism

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Naturally occurring glucokinase mutations at the same amino acid residue cause opposite clinical phenotypes of hypo- and hyperglycaemia

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Background and aims: Glucokinase (*GCK*) mutations cause disorders of glucose homeostasis. Heterozygous activating and inactivating mutations cause hyperinsulinaemia of infancy (HI) and maturity-onset diabetes of the young (MODY) respectively. Activating mutations cluster at the allosteric activator site, a target for pharmacological GCK activators (GKAs) which are currently in development. This study reports 2 novel activating mutations (V389L & T103S), at residues where GCK-MODY mutations (V389D & T103N) have also been reported. We hypothesised that the physiochemical properties and position of the substituted residue could result in opposite phenotypes. We structurally and functionally characterised these mutations in the presence and absence of GCK regulators, providing further insight into critical residues within this important drug target.
Materials and methods: Recombinant human wild-type (WT) GCK and all 4 mutants were generated. Enzyme kinetic activity was determined spectrophotometrically using an $NADP^+$-coupled assay. Kinetic characteristics and responses to the physiological inhibitor glucokinase regulatory protein (GKRP) and pharmacological GKA RO0281675 were calculated. Structural modelling and bioinformatic analysis were performed using PyMOL.
Results: Both HI mutations were kinetically activating with relative activity indices (RAI) of 6.0 and 8.4 for V389L and T103S respectively (vs 1.0 for WT). This higher activity was driven by an increased affinity for glucose (glucose $S_{0.5}$ 3.5 ± 0.1 for V389L, 3.3 ± 0.1 for T103S, 7.5 ± 0.1mM for WT). The MODY mutation V389D had dramatically decreased enzyme activity (RAI <0.1). Paradoxically the T103N MODY mutation was mildly kinetically activating ($S_{0.5}$ 5.8 ± 0.1mM, K_{cat} 51.5 ± 1.1S^{-1}, RAI 1.5). Inhibition assays showed a similar response to GKRP for WT and V389L (IC_{50} both 1.1 ± 0.1 GKRP units), whilst T103S and -N had decreased affinity (1.5 ± 0.1 and 1.9 ± 0.1 respectively). All mutations responded to the GKA; T103N had a similar fold increase in $K_{cat}/S_{0.5}$ to WT (18.0 ± 1.5 vs 14.4 ± 0.3 respectively), whilst that for T103S & V389L was lower (5.7 ± 0.5 and 7.4 ± 1.3 respectively). V389D $K_{cat}/S_{0.5}$ increased considerably (33.9 ± 2.1). V389 lies within an α-helix, with both mutations causing extra intra-helical bonds. T103 is in a β-sheet close to the allosteric activator site, both mutations losing an intra-strand bond whilst forming new polar interactions.
Conclusion: Different physiochemical properties of substituted residues within GCK can determine clinical phenotype. V389 (or its α-helix) is integral to protein activity, with its mutation affecting glucose turnover. T103 lies in a β-sheet close to the allosteric activator site. Both T103S/N have increased GCK activity, suggesting the pathogenesis for T103N may be due to catalytic instability (reflected by decreased GKRP affinity). Our study has implications for drug development, as it demonstrates a fine balance between catalytic and structural stability upon intervention at certain GCK residues/areas of GCK secondary structure.
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Identification of an ubiquitin-like domain as a potential new interaction partner of glucokinase in pancreatic beta cells

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Background and aims: In pancreatic beta cells, glucokinase (GK) acts as a glucose sensor and catalyzes the rate-limiting step for initiation of glucose-induced insulin secretion. GK is mainly regulated on the posttranslational level by protein-protein interactions. The bifunctional enzyme 6-phosphofructo-

2-kinase/fructose-2,6-bisphosphatase serves as an activating GK interaction partner in both, beta cells and liver. In contrast an inhibiting GK protein, the GK regulatory protein is only expressed in liver, but not in beta cells. This raised the question whether unidentified beta cell specific GK interaction partners exist. Therefore the aim of this study was to identify beta cell specific GK interaction partners through a yeast two hybrid (YTHS) screening of a rat islet library.
Materials and methods: RNA isolated from rat pancreatic islets was used to generate a cDNA library by SMART technology (Clontech). Recombination of the cDNA library with the pGADT7-R vector was performed in S. cerevisiae AH109. Mating with the pGBKT7-GK containing S. cerevisiae Y187 yeast strain was established resulting in analysis of 5.5 x 10^5 clones in three independent runs. Initially positive clones were characterized by histidine (His) and adenine (Ade) reporter gene assays in yeast. Thereafter binding to GK was determined in MIN6 beta cells using a recently established fluorescence based mammalian two-hybrid system (MMTHS) in a high-throughput scanR (Olympus) microscopy setup.
Results: 73 positive clones were selected by His reporter gene expression in yeast, whereas four clones showed also significant Ade reporter gene expression. In YTHS experiments with the control plasmid lamin only one clone exhibited specific interaction with GK. Sequence and protein database analyses revealed a 180 amino acid containing protein fragment with homology to the N-terminal part of midnolin 2 of mus musculus. Thus, no full-length protein was discovered. However, a complete sequence of an ubiquitin-like domain (UbD) consisting of 72 amino acids was identified inside of the protein fragment by NCBI-BLAST data base analyses and further analyzed in the MMTHS. For this the UbD was subcloned in frame with the binding domain (pBIND-ECFP-UbD), and interaction with GK subcloned in frame with the activation domain (pACT-GK) in comparison to control (pACT) was elucidated in single MIN6 beta cells. Interestingly, a specific interaction between the UbD and GK determined as an increase of the EYFP reporter gene expression in relation to the constitutively expressed ECFP fluorescence over time was elucidated.
Conclusion: We have identified an ubiquitin-like domain as a potential new GK binding partner. Recently an ubiquitin interacting motif has been discovered in GK. As the ubiquitin-proteasome pathway plays an important role in pancreatic beta cells our study supports the hypothesis of GK regulation by ubiquitination.

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The novel naturally occurring activating glucokinase gene mutation E442K maintains INS-1E cell proliferation and protects against glucose-induced apoptosis
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Background and aims: In addition to its glucose sensor function in islet beta cells, glucokinase (GK) has recently been proposed to participate in cell proliferation and survival. These GK-mediated effects appear to require IRS-2 and BAD, respectively. We have previously described in humans two novel mutations located almost contiguously in the same domain of GK yet having opposite clinical phenotypes: a naturally inactivating (*GCK*-E440G) and activating *(GCK*-E442K) GK mutations which cause monogenic diabetes and congenital hypoglycaemia, respectively. In order to establish a potential link between GK activity and beta cell survival as well as expansion, we constitutively expressed these mutant variants in the insulinoma INS-1E cell line.
Materials and methods: Using lentiviral technology, we created stable clones of INS-1E cells constitutively expressing GK (INS-1E-cmv.GCK-WT.ires.GFP), the two mutant variants (INS-1E-cmv.GCK-E440G.ires.GFP, INS-1E-cmv.GCK-E442K.ires.GFP) or the control GFP lentiviral vector (INS-1E-cmv.ires.GFP). Transcript levels for GK, E442K, E440G, GFP, BAD and IRS2 were determined by quantitative real time RT-PCR (QT-PCR). Acute insulin secretion in response to glucose (3 and 15 mM) was also measured. In addition, the impact of increasing concentration of glucose (3, 11 and 20 mM) on proliferation and survival was estimated at 72 hours.
Results: Overexpression of GK or E442K in INS-1E did not alter glucose-induced insulin secretion (GSIS) as compared to control GFP cells. In contrast, overexpression of the inactivating mutation E440G resulted in impaired GSIS. QT-PCR revealed that both the GK and E442K transcript were expressed at similar levels whereas E440G was at least 5-fold higher, explaining the potential altered GSIS. Similar results were found with GFP transcript levels. Interestingly, BAD and IRS2 expression levels remained unaltered in all clones as compared to control GFP cells. More importantly, our results showed that after 72 hours at either 3 mM or 20 mM glucose, the clone expressing the E442K variant displayed significantly reduced apoptosis (12- and 6-fold, respectively) whereas the E440G only protected cells at low (2-fold) but not at high glucose as compare to GFP expressing cells. GK had no protective effect. In contrast all clones exhibited protection at 11 mM glucose (6-fold as compared to GFP), corresponding to normal culture conditions. Proliferation at 11 or 20 mM glucose was identical for all clones. Astonishingly, the E442K clone was also able to sustain proliferation even at 3 mM glucose.
Conclusion: Taken together, our results indicate that the naturally occurring activating mutation GK-E442K may convey protection and maintain proliferation of β-cells under altered glycaemic conditions. Yet, the fact that the inactivating mutation GK-E440G, located in the vicinity of the E442K, also protects at low glucose suggests an insulin independent effect. We are currently investigating a potential link between the BAD protein and these mutant variants in β-cell survival.
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Activation of PPARδ promotes mitochondrial energy metabolism and decreases basal insulin secretion in palmitate-treated beta cells
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Background and aims: The peroxisome proliferator-activated receptor δ(PPARδ)regulates the expression of genes involved in cellular lipid and cell energy metabolism in many metabolically active tissues, such as liver, muscle and fat and plays a role in the cellular response to stress and environmental stimuli. The particular role of PPARδ in insulin-secreting beta-cells, however, is not as well understood; therefore, our aim was to investigate the cell-specific role of PPARδ on mitochondrial energy metabolism and insulin secretion in beta-cells.
Materials and methods: After exposing a Syrian hamster pancreatic beta-cell line, HIT-T15, to high-concentrations of palmitate and/or the specific PPARδ agonist GW501516, we detected the gene expression changes for transcripts associated with mitochondrial biogenesis, such as peroxisome proliferator-activated receptor gamma co-activator 1 (PGC-1α), nuclear respiratory factor 1 (NRF-1), mitochondrial transcription factor A (mtTFA), using real-time quantitative polymerase chain reaction (RTQ-PCR). The protein levels of the mitochondria uncoupling protein 2 (UCP2) were measured by western blot analysis; the insulin secretion capacity and ATP/ADP ratio were analyzed using enzyme linked immunosorbent assay (ELISA) and high performance liquid chromatography, respectively.
Results: Activation of PPARδ promoted generation of mitochondrial ATP, as well as expression levels of PGC-1α, NRF-1 and mtTFA in palmitate-treated HIT-T15 cells. Activated PPARδ also decreased basal insulin secretion, but had no effect on glucose-stimulated insulin secretion (GSIS) via increased amounts of UCP2.
Conclusion: GW501516 treatment enhanced mitochondrial energy metabolism, but it also promoted a concomitant mitochondrial uncoupling and resulted in decreased basal insulin secretion and restricted GSIS. Activation of PPARδ induced a reduction of basal insulin secretion, but did not appear to improve GSIS; this observation indicated the possible action of a protective mechanism responding to the alleviation of basal insulin load in lipotoxic beta-cells.

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Role if tissue specific alterations of mitochondrial dynamics in development of type 2 diabetes
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Background and aims: Mitochondrial dysfunction has been proposed to play an important role in the development of type 2 diabetes. Mitochondrial function is regulated in a tissue specific manner and in recent studies mitochondrial dynamics have been elucidated to be important to maintain cellular metabolism. Mitochondria constitute a mobile network that continuously

cycle through fusion and fission events. By this process dysfunctional mitochondria are segregated and their removal by autophagy is initiated. Mitofusin 1 and 2 (Mfn1 and Mfn2) and the optic atrophy 1 (Opa1) are essential for mitochondrial fusion, whereas the fission protein 1 (Fis1) and the dynamin related protein 1 (Drp1) control fission. Alterations in mitochondrial function may be evoked by obesity, and thus, crucial to explain both insulin resistance in peripheral tissue and pancreatic beta cell dysfunction resulting in impaired insulin secretion in type 2 diabetes. Therefore the aim of this study was to investigate tissue specific alterations in mitochondrial dynamics in obese ob/ob mice compared to normal weight control mice.
Materials and methods: Islets from obese ob/ob mice (B6.V-lebob) and control mice (C57BL/6J) were isolated by collagenase digestion. Furthermore liver, muscle, adipose tissue and brain were taken from ob/ob and control mice. RNA and protein were isolated and quantitative Real-Time PCR analyses and western blot analyses of Fis1, Drp1, Opa1, Mfn1 and Mfn2 were carried out, respectively. Additionally frozen sections were made for immunofluorescence analyses.
Results: Gen expression of Fis1, Drp1, Opa1, Mfn1 and Mfn2 in islets of obese ob/ob mice was significantly reduced compared to control mice and the resulting expression pattern indicated an imbalance of mitochondrial fusion and fission processes. In liver Fis1 was down regulated in ob/ob mice compared to control, while Drp1 was up regulated. As both proteins cannot replace each other, fission was disturbed in liver of ob/ob mice. Furthermore immunofluorescence analyses revealed an inhomogeneous expression pattern of fission proteins in liver of ob/ob mice. Additionally Mfn1 and Mfn2 were significantly reduced on the gene as well as protein level in liver of ob/ob mice. Interestingly Mfn2 a protein which plays a role in mitochondrial substrate oxidation was significantly down regulated in islets, liver, muscle and adipose tissue, but not in the brain of ob/ob mice compared to control. Gene and protein expression of Fis1, Drp1, Mfn1 and Mfn2 was reduced in adipose tissue and muscle of ob/ob mice compared to control, but in muscle to a much higher extent. Solely in muscle a significant down regulation of the Opa 1 protein was determined.
Conclusion: We have observed tissue specific changes in mitochondrial dynamic in obese mice compared to normal weight control mice. In future experiments we will elucidate whether these alterations are a reversible adaptive process maintaining cell function or represent mitochondrial dysfunction. In conclusion, our study provides further evidence that obesity induced mitochondrial alterations contribute to the development of type 2 diabetes.

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Implication of mitochondria in IAPP induced-beta cell toxicity
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Background and aims: Type 2 diabetes is characterised by islet dysfunctions that lead to the impairment of insulin secretion. The presence of islet amyloid deposition is a recognised hallmark in islets of type 2 diabetes patients. Amyloid fibrils are formed by human islet amyloid polypeptide (hIAPP). There is now very strong evidence supporting the key role of amyloidogenesis in the progressive loss of pancreatic beta-cell mass and function. Mitochondria have a central role in the regulation of insulin secretion since the latter is largely controlled by ATP production through oxidative phosphorylation (OXPHOS) taking place in the mitochondrial respiratory chain. The main objective of this project is to investigate whether mitochondria may play a role in the mechanisms by which human IAPP induces beta-cell cytotoxicity.
Materials and methods: The rat pancreatic beta-cell line INS1E was stably transfected with a *hIAPP* plasmid (hIAPP cells) or pcDNA3 (control cells). In order to characterise the impact of hIAPP on beta-cell function, we measured insulin and IAPP secretion and intracellular calcium mobilisation in response to glucose. Calcium levels were measured using Fura-2 labelling. Mitochondrial function was explored by monitoring cellular respiration, mitochondrial membrane potential and reactive oxygen species (ROS) production. Rhodamine 123 and CM-H2DHCFA probes were used to monitor membrane potential and ROS levels, respectively.
Results: In response to 16,7 mM glucose, insulin and IAPP secretion was strongly decreased in hIAPP compared with control cells (3.5 ± 0.7 *vs* 15.6 ± 2.9 % insulin release expressed as a percentage of cellular insulin content and 2.6 ± 0.9 *vs* 8.7 ± 1.4 % IAPP release expressed as a percentage of cellular IAPP content, p<0.01). Consistent with these results, the study of calcium signals demonstrated an absence of response to glucose in hIAPP cells. Whereas 76,61% of control cells (194/201) exhibited a normal response to 16.7mM glucose, none of the hIAPP cells tested were able to respond to glucose (0/192). hIAPP cells showed an oxygen consumption higher than control cells which is likely due to an increase in uncoupled respiration (25.8 ± 2.2 vs 15.6 ± 2.5 pmol/s/million of cells, p<0.05). Moreover, hIAPP cells exhibited a significantly increased mitochondrial membrane potential relative to control cells which is represented by a greater decrease of probe fluorescence in response to glucose stimulation (64.02 ±1.45 vs 79.57 ± 1.73 % leftover fluorescence relative to 2.2 mM glucose, p<0.05). In addition, hIAPP cells cultured in 16.7 mM glucose for 24h had significantly elevated ROS levels compared to control cells cultured in the same conditions (1.37 ± 0.1 vs. 1.01 ± 0.02 fold over 2.2 mM glucose, p<0.01).
Conclusion: Our preliminary results show that hIAPP overexpression decreased glucose stimulated insulin and IAPP secretion. Those results could be explained by an inhibition of intracellular calcium mobilisation, an increased ROS generation in response to glucose, and an altered mitochondrial function.
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PS 30 Cytokines and beta cell survival

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Mimitin overexpression protects insulin-producing INS1E cells against cytokine-induced apoptosis via prevention of MAP1S action

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Background and aims: Mimitin is a mitochondrial protein, which was shown to be induced by cytokines in insulin-producing cells. It has been reported that in some cell types it can interact with a partner protein called MAP1S. MAP1S is a microtubule associated protein, which triggers a cytoplasmic signal for apoptosis, mainly via induction of ER stress. The aim of this study was to investigate the possible interaction of these two proteins in insulin-producing cells and the influence of mimitin overexpression on cytokine-stimulated apoptotic pathways.
Materials and methods: Insulin-producing INS1E cells and INS1E-hMim cells (overexpressing mimitin) were used. The cells were treated with IL-1 beta 600 U/ml or with a cytokine mixture (60 U/ml IL-1 beta, 175 U/ml TNF-alpha and 14 U/ml IFN-gamma) for 24 h. Cell viability was estimated by MTT assay, cell proliferation by BrdU incorporation, NF-kappa B by an SEAP reporter gene assay, nitrite accumulation was determined by the Griess method and caspase activation was quantified by flow cytometry. Fluorescent microscopy was used to analyze mimitin and MAP1S expression.
Results: The analysis of mimitin and MAP1S expression using specific fluorescent probes revealed that mimitin resided in the mitochondria, while MAP1S was in the cytoplasm. Upon exposure to cytokines mimitin partially moved from mitochondria into the cytoplasm and interacted there with MAP1S. Overexpression of mimitin protected INS1E cells against cytokine toxicity and prevented deleterious effects of cytokines towards cell proliferation (MTT 24h: INS1E IL-1 beta 58%, cytokine mix 37% vs. INS1E-hMimitin IL-1 beta 84 %, cytokine mix 57%; proliferation rate after 24h: INS1E IL-1 beta 58%, cytokine mix 28% vs. INS1E-hMimitin IL-1 beta 85%, cytokine mix 54%). The cytokine-induced caspase-3 activation was completely abolished in INS1E-hMim cells (24 h, INS1E: IL-1 beta 156 ± 16, cytokine mix 165 ± 19; INS1E-hMim: IL-1 beta 110 ± 11, cytokine mix 88 ± 10). The mitochondrial caspase-9 activation induced by cytokines was not affected by mimitin overexpression (24 h, INS1E: IL-1 beta 188 ± 16, cytokine mix 181 ± 16; INS1E-hMim: IL-1 beta 129 ± 5, cytokine mix 150 ± 11). Interestingly, caspase-8, triggering the extrinsic apoptotic pathway and caspase-12, associated with the ER stress response, were both downregulated by mimitin overexpression (caspase 8 after 24 h, INS1E: IL-1 beta 187 ± 14, cytokine mix 138 ± 8; INS1E-hMim IL-1 beta 110 ± 8, cytokine mix 108 ± 8; caspase-12, INS1E: IL-1 beta 151 ± 11, cytokine mix 198 ± 17; INS1E-hMim: IL-1 beta 105 ± 10, cytokine mix 116 ± 15). No significant effect of mimitin overexpression on the NF-kappa B-iNOS pathway was observed.
Conclusion: Mimitin prevents cytokine-induced death of insulin-producing cells and stimulates cell proliferation. We hypothesize that the protective effect of mimitin against cytokine-induced apoptosis occurs via prevention of cytotoxic action of the proapoptotic protein MAP1S.

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Expression of iNOS in pancreatic islets of human type 2 diabetic patients

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Background and aims: Accumulating data suggest that nitric oxid (NO) produced by inducible NOS (iNOS) play an essential role in the β-cell dysfunction and apoptosis. The main purpose of this investigation was to clarify whether the iNOS derived NO is involved in secretory defect and β-cell dysfunction of pancreatic islets isolated from human type 2 diabetic patients is associated with exaggerated NO production through the induction of iNOS expression.
Materials and methods: iNOS expression was analyzed by confocal microscopy, Western blot and qPCR in islets from both human type 2 diabetic and non-diabetic donors. Hormone secretion was determined with RIA.
Results: Confocal microscopy revealed that iNOS is expressed in pancreatic islet cells (insulin, glucagon and somatostatin) of type 2 diabetic subjects and that no iNOS was detected in islets from normal human donors. iNOS mRNA and protein expression was markedly higher in diabetic vs normal human islets ($p<0.001$). The hormone secretory pattern of type 2 diabetic pancreatic islets incubated at different glucose concentrations showed a reduced insulin and somatostatin response to glucose. The suppressing effect of glucose on glucagon secretion was not observed in the incubated islets of type 2 diabetic subjects.
Conclusion: Our data shows that long term hyperglycemia seen in human type 2 diabetic patients, based on their HbA1c, might result in the expression of iNOS in the pancreatic islets which consequently might disrupt he normal β-cell function.
Supported by: Swedish Science Council

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Mcl-1 degradation by pro-inflammatory cytokines and palmitate is an early and major event for beta cell apoptosis

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Background and aims: Apoptosis of the insulin-secreting pancreatic beta cell is a key feature of diabetes mellitus and the mitochondrial pathway of apoptosis is a major mediator of beta cell death. The myeloid cell leukemia sequence 1 (Mcl-1) is an important anti-apoptotic protein of the Bcl-2 family, a group of proteins involved in the mitochondrial pathway of apoptosis. The aim of this study was to evaluate the role of Mcl-1 in beta cell apoptosis.
Materials and methods: We studied the effect of pro-inflammatory cytokines (IL-1β or TNF-α, in combination or not with IFN-γ), free fatty acids (palmitate or oleate), and chemical ER stressors (CPA, thapsigargin, tunicamycin) on Mcl-1 mRNA and protein expression in INS-1E cells, and, in selected experiments, in FACS-purified rat beta cells. Using mRNA silencing and recombinant adenoviruses, we studied the effect of Mcl-1 knockdown and overexpression on beta cell function and apoptosis. Expression of Mcl-1 and key downstream genes and proteins were measured by real time RT-PCR and/or Western blot. Localisations of Bax and cytochrome c were studied by immunofluorescence in cells overexpressing Mcl-1 and treated with cytokines, palmitate or thapsigargin. Cell viability was evaluated by HO 342 and propidium iodide.
Results: All cytotoxic stresses described above increased Mcl-1 mRNA expression by 2 fold, but rapidly and preferentially decreased Mcl-1 protein expression by 30 to 60% ($p< 0.05$). ER stress-induced Mcl-1 down-regulation was prevented upon PERK knock down and resulting inhibition of eIF2α phosphorylation, indicating that translation arrest contributes to Mcl-1 downregulation. Additionally, the effect of cytokines on Mcl-1 expression was also partially prevented in presence of a JNK inhibitory peptide. Knocking-down Mcl-1 using siRNAs increased by 40% apoptosis and caspase 3 cleavage induced by cytokines, palmitate or thapsigargin ($p<0.01$). On the other hand, Mcl-1 overexpression reduced by 50% Bax translocation to the mitochondria, cytochrome c release, caspase 3 cleavage and apoptosis induced by the beta cell death effectors ($P< 0.01$). Neither Mcl-1 knockdown nor overexpression modified basal or glucose-stimulated insulin secretion.
Conclusion: The present data suggest that Mcl-1 downregulation is a crucial event leading to beta cell apoptosis and provide new insights in the mechanisms linking ER stress and the mitochondrial intrinsic pathway of apoptosis. Mcl-1 is therefore an attractive target for the design of new strategies in the treatment of diabetes.
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Effects of proinflammatory cytokines on the pancreatic alpha cells response to glucose, Zn^{2+} and insulin

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Background and aims: The study of the effects of diabetogenic factors on the pancreatic α-cell are relatively limited when compared to its neighbour, the pancreatic β-cell. In recent years the regulation of glucagon secretion has attracted considerable attention from molecular bioscientists. The dysregulation of glucagon secretion in both type 1 and type 2 diabetes mellitus is of physiologic and pathologic importance as little has been achieved in relation

to counteracting the dysregulation of glucagon secretion in diabetes. The effects of proinflammatory cytokines on the β-cell are well documented but little has been reported on the mechanisms of cytokine induced α-cell dysregulation. IL-1β, TNF-α and IFN-γ are commonly used for initiation of dysfunction and death in the pancreatic β-cell. This investigation has determined the effects of these cytokines on the pancreatic α-cell under various incubation conditions relating to the availability of islet cell secretory products.
Materials and methods: The clonal cell line α-TC1-9 were seeded in 6 well plates under various conditions in relation to proinflammatory cytokine levels, Zn^{2+} levels, Insulin Levels and glucose levels for various time points. Metabolite consumption, Glutathione ratio and ATP levels were assessed using enzymatic methods, glucagon levels were assessed using HTRF techniques and Ca^{2+} fluctuations were assessed using flow cytometry.
Results: Addition of various concentrations of the cocktail of proinflammatory cytokines to the αTC1-9 cell line (various dilutions of the following: 5U/ml IL-1β, 1000U/ml TNF-α and 500U/ml IFN-γ) resulted in a dose dependent increase in glucagon secretion, up 327% compared to basal levels (n=6). Intracellular levels of reduced glutathione were increased by 1.2 fold, while intracellular levels of oxidised glutathione were not increased significantly in any condition. Glucose and Glutamine consumption was also increased, dose dependently, with increases in proinflammatory cytokine cocktail concentration. Glucagon secretion was reduced by addition of Zn^{2+} and/or insulin an effect partly attenuated (34.3-69.4%) by addition of various concentrations of glucose (0-25mM). This inhibitory effect was lost in a dose dependent fashion with the addition of the proinflammatory cytokine cocktail.
Conclusion: Proinflammatory cytokines had a dose dependent effect on metabolite consumption including glucose, glutamine etc. and glucagon secretion from α-TC1-9 cells. Glucose was able to partly attenuate the negative effect of the cytokines.
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Toll-like receptor 4 in islets of Langerhans - a potential role in beta cell death in type 2 diabetes
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Background and aims: Type 2 diabetes is a characterised by insulin resistance, beta-cell dysfunction and increased systemic levels of free fatty acids (FFA). Furthermore, it is recently becoming clear that activation of the innate immune system via toll-like receptors (TLRs), in particular TLR2 and TLR4, is implicated in the pathogenesis of insulin resistance and type 2 diabetes. Activation of TLR4 by ligands of both exogenous and endogenous origin (e.g. LPS, FFA) causes an initiation of intracellular pro-inflammatory signalling pathways and a subsequent release of pro-inflammatory mediators such as TNFα, IL-1 and IL-6. Most cells in the body, including immune and insulin-sensitive cells, have a low basic expression of TLR4 under normal conditions. In obese and type 2 diabetic patients the level of the TLR4 ligands LPS and FFA are elevated, and recent studies demonstrate a resulting increase in TLR4 expression in macrophages, adipocytes and muscle cells suggesting a link between TLR4 expression and development of insulin resistance. In addition to insulin resistance, type 2 diabetes is characterised by beta-cell dysfunction. Recent studies report increased infiltration of macrophages in islets of Langerhans from patients with type 2 diabetes and in a variety of type 2 diabetic animal models, raising the possibility that TLR4 expression and a resulting cytokine release might be increased in islets under diabetic conditions. TLR4 expression has been described in pancreatic islets under normal conditions; however changes in the expression pattern have not yet been investigated with respect to diabetes development. Our hypothesis is that TLR4 expression and signalling is increased in islets of Langerhans in obesity and the resulting increase in secretion of cytokines causes beta-cell dysfunction and manifestation of diabetes. We investigate the hypothesis in the db/db mouse - an animal model of type 2 diabetes.
Materials and methods: Islets of Langerhans from male db/db (4, 8 and 15 weeks old, representing diabetes progression with age) as well as control db/+ (15 weeks old) mice were isolated using collagenase digestion. RNA was extracted and changes in gene expression of Toll-like pathway products (TLR4, TLR2, MyD88, NFκB) and selected cytokines (TNFα, IL-1α, IL-1β, IL-6, IP-10, IFNγ) during development of diabetes examined using real-time PCR. Additionally, secretion over a 24h period of the above mentioned cytokines from islets was quantified (Milliplex).
Results: TLR4 mRNA was found in islets from both lean and obese mice with a 7.4 ± 1.6 fold higher level in islets from obese diabetic 15 weeks old db/db compared to 15 weeks old db/+ mice ($p<0.01$) ($n\geq3$). TLR4 gene expression increased with progression of diabetes in db/db mice showing a 5.6 fold higher level of TLR4 mRNA in islets from 15 weeks old db/db compared to islets from 4 weeks old db/db mice ($p<0.01$) ($n\geq3$). Furthermore, both protein and gene levels of all cytokines examined, except IFNγ, increased significantly in islets during development of type 2 diabetes in db/db mice. No significant differences were observed for IFNγ.
Conclusion: Expression of TLR4 and its intracellular signalling molecules in db/db mouse islets were increased in parallel with diabetes development. Also, expression and secretion of cytokines and chemokines were found significantly increased in diabetic vs. non-diabetic mouse islets. Our results support the hypothesis that, in addition to its contribution to insulin resistance, TLR4 may also be a causative factor in beta-cell death leading to type 2 diabetes.

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Regulation of beta cell survival and function by 14-3-3 proteins
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Background and aims: Diabetes is associated with increases and decreases in pancreatic beta cell death and function. Numerous factors promote cell survival through the activation of survival kinases and deactivation of pro-apoptotic proteins. Previously, we have demonstrated that insulin promotes beta cell survival and proliferation via Raf-1 kinase. The importance of Raf-1 as a regulator of survival was further elucidated by pharmacological inhibition of Raf-1, which induced cell death. In other cell types, scaffold proteins of the 14-3-3 family regulate Raf-1 and other growth factor signaling cascades, but their function in beta cells is unknown. We, therefore, hypothesize that 14-3-3 proteins regulate beta cell function and survival.
Materials and methods: The MIN6 beta cell line and primary mouse islets were used to assess the role of 14-3-3 proteins on cell function and survival by overexpression of proteins and shRNA. Quantitative PCR (qPCR) was performed to measure mRNA levels. Fluorescent microscopy was used to visualize protein localization as well to quantify cell death. Western blots and co-immunoprecipitation were used to detect protein interactions, phosphorylation, and expression. Insulin was measured by ELISA.
Results: Of the 7 isoforms, 14-3-3 ζ, η, θ transcripts levels were expressed at the highest levels, followed by γ, β, ε, and σ in MIN6 cells and mouse islets. Fluorescent microscopy showed that 14-3-3 ζ was localized to beta cells, endothelial cells, but not exocrine tissue in the mouse pancreas. In isolated primary beta cells, 14-3-3 ζ was predominantly cytoplasmic, and co-localization with insulin granules was also observed. To analyze the function of 14-3-3 proteins, difopein-EYFP, a pan 14-3-3 inhibitor, was expressed in MIN6 cells. Inhibition caused a 3.8-fold increase ($p<0.05$) in propidium iodide-positive cells, a marker of cell death and a 1.5-fold increase ($p<0.05$) in cleaved caspase-3, a marker of apoptosis. Moreover, there was a 1.3-fold increase ($p<0.05$) in basal insulin release. As several studies have shown that 14-3-3 ζ regulates cell survival, MIN6 cells were transfected with wild-type (WT) 14-3-3 ζ or control plasmids. Treatment with cytokines (25ng/ml TNF-α, 10ng/ml IL-1β, and 10mg/ml IFN-γ) induced a 2.5-fold increase in cell death ($p<0.05$), which was completely prevented by WT-14-3-3 ζ over-expression. Similarly, cell death stimulated by 1μM thapsigargin was reduced by 43% ($p<0.05$). Transfection of MIN6 cells with shRNAs against 14-3-3 ζ led to a 1.4-fold increase ($p<0.05$) in cleaved caspase-3 levels. Dissociation of Raf-1 from 14-3-3 ζ is required for its activation, and treatment of MIN6 cells with a picomolar concentration of insulin decreased by 63% ($p<0.05$) the amount of Raf-1 that complexed with 14-3-3 ζ. The decrease in 14-3-3 ζ: Raf-1 association was mirrored by an increase in 14-3-3 ζ association with Bad, a proapoptotic protein. Static cultures on MIN6 cells were used to assess the effect of WT-14-3-3 ζ or shRNAs on insulin secretion. Overexpression of WT-14-3-3 ζ induced an 8.5-fold increase in basal insulin secretion from MIN6 cells. Conversely, shRNA-mediated knockdown of 14-3-3 ζ decreased basal and glucose-induced insulin secretion.
Conclusion: The present study demonstrates for the first time that 14-3-3 proteins regulate beta cell survival and function. Alterations in the expression of 14-3-3 proteins may represent a novel therapeutic approach to modulate insulin signaling and promote beta cell survival and function.
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Expression of salivary type amylase in human pancreatic beta cells and its induction by pro-inflammatory cytokines in MIN6 beta cells

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Background and aims: Autoantibodies against amylase (AMY) were detected at a high frequency in sera from patients with diabetes associated with autoimmune pancreatitis (DAIP) or with fulminant type 1 diabetes (FT1DM). The possible relationship between beta-cell destruction and autoimmunity against AMY is not clear. The aim of this study was to examine AMY expression in 1) the pancreatic islet-cells of autopsied patients with FT1DM and 2) mouse MIN6 beta cells exposed to Th1 cytokines.
Materials and methods: AMY expression in islet beta cells from three FT1DM patients dead 2-5 days after the onset and three non-diabetic control pancreas were analyzed by immunohistochemical staining with AMY1 (salivary type) -specific monoclonal and AMY2 (pancreatic) -specific monoclonal antibodies. Quantitative RT PCR was used to detect the expression of both AMY1 and AMY2 (pancreatic type) in MIN6 beta cells incubated with Th1 cytokines. The MIN6 cells were also subjected to immunohistochemical staining using monoclonal AMY1 antibodies.
Results: Severe mononuclear cells infiltration (insulitis) was observed in islets of FT1DM patients but not the islets of control non-diabetic subjects. An AMY1, but not AMY2, monoclonal antibody identified immunoreactivity of AMY in beta cells, but not alpha or exocrine acinar cells, of non-diabetic human. The AMY1 staining was also observed in the islets from FT1DM patients. Western blotting revealed that serum from patients with autoimmune pancreatitis had IgG detecting AMY1. AMY1 mRNA levels in MIN6 beta cells were 108-fold higher than AMY2. MIN6 beta cells cultured with either IFN-γ or TNF-α alone did not affect AMY1 expression. In contrast, simultaneous addition of IFN-γ and TNF-α induced a synergistic 5-fold increase of AMY1 but not AMY2 levels. The MIN6 beta cells cultured simultaneously with IFN-γ and TNF-α showed an increased AMY immunoreactivity compared to the cells treated with IFN-γ or TNF-α alone.
Conclusion: We conclude that human pancreatic islet beta-cells express AMY1 and its expression was also observed in islets from FT1DM patients. Infiltrating mononuclear cells in FT1DM or DAIP are known to produce Th1 cytokines. It was of interest that the expression of AMY1 gene in pancreatic MIN6 beta-cells was enhanced synergistically by Th1 cytokines. This observation suggests that islet beta-cells hyper-expressing AMYI may be the target for beta-cell autoimmunity in DAIP and FT1DM.

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Siglecs are differentially expressed in pancreatic islets and regulate beta cell function and survival

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Background and aims: In both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), local inflammation and cytokine and chemokine production within pancreatic islets has been observed and is detrimental for the β-cell. Siglecs (Sialic-acid binding immunoglobulin like lectins) are cell surface receptors expressed on haematopoietic cells which participate in immune responses. Their prominent position, negative charge and widespread distribution make sialic acids an important component in cell-cell interactions as well as pathogen and toxin binding. Our investigations revealed a cell-type specific expression of Siglecs in pancreatic islets, which is altered in diabetic conditions, hinting towards their role in survival and function of these pancreatic cells.
Materials and methods: The expression of Siglecs 3, 5, 6, 7, 8 and 10 in pancreatic sections was analyzed by immunofluorescence in pancreatic sections from autopsy from patients with T2DM and controls as well as in isolated human islets exposed to a diabetic milieu (increasing glucose (11.1-33.3 mM), 2 ng/ml IL-1β alone or in combination with 1,000 U/ml IFN-γ, 100 ng/ml LPS, 0.5 mM palmitate, 50 μg/ml synthetic ds RNA analogue Poly:IC or 50 μM H_2O_2). The siglecs cellular localization was confirmed by Confocal Laser scanning microscopy. Siglec expression was evaluated by quantitative real time PCR of cDNA from human pancreases from autopsy. To investigate its role in β-cell function, Siglec 7 over-expression by plasmid transfection was carried out followed by glucose stimulated insulin secretion (GSIS).
Results: Triple staining for Siglecs 3, 5, 6, 7, 8 and 10; glucagon and insulin in pancreatic sections of healthy controls and patients with T2DM showed cell type specific siglec expression in the human pancreas: Siglecs 3, 5 and 8 were expressed in the glucagon producing α-cells, Siglecs 7 and 10 were expressed in the insulin producing β-cells and Siglec 6 was expressed in the exocrine pancreas. Siglec expression was not uniform in the cells but was aggregated at certain locations; this was observed independently from diabetes. This expression pattern hints towards a cell-cell interaction junction which is facilitated by these siglecs. A diabetic milieu had an inductive effect on siglec expression in those cells in human islets *in vitro* and *in vivo*. Real time PCR analysis revealed that siglecs expressed in α-cells Siglec-3,-5 and -8 showed a 5.9-fold, 7.9-fold and 6.1-fold increase in its expression in patients with T2DM as compared to the non diabetic controls. In contrast, in the siglecs expressed in the β-cells, there was an 85% decreased expression in siglec 7 and a 47% decrease in siglec 10 expression in patients with T2DM. Since Siglec 7 was markedly downregulated in T2DM in poorly functional β-cells, we wanted to know if over-expression of siglec 7 has an opposite effect on β-cell function. Indeed, plasmid over-expression of Siglec 7 increased GSIS 1.5-fold in human islets and prevented glucose and palmitate induced apoptosis, when compared to the lacZ transfected control islets.
Conclusion: Our data suggest that Siglecs are differentially expressed in pancreatic islets, are regulated in T2DM and influence β-cell function and survival. Siglecs could play an important role not only in the crosstalk of β-, α- and immune cells but also in maintaining glucose homeostasis.

PS 31 Apoptosis of beta cells

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MST1 mediates beta cell apoptosis and impaired function

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Background and aims: Pancreatic β-cell death is the fundamental cause of type 1 and type 2 diabetes (T2DM). Mammalian sterile 20-like kinase 1 (MST1) is a serine threonine kinase, which mediates apoptosis in response to cytotoxic stress. MST1 is both, cleaved and activated by caspases, and also serves as an activator of caspases to amplify the apoptotic signaling pathways. In the present study, we investigated the possible patho-physiological activation of MST1 in β-cells under diabetic conditions, which may be a major common pathway of β-cell death in diabetes.
Materials and methods: Isolated human islets and the human β-cell line CM9 were exposed to a diabetic milieu (cytokine mixture IL-1β/IFNγ, oxidative stress (H_2O_2) or increasing glucose concentrations). Phospho-MST1 and MST1 cleavage, Phospho-JNK, Phospho-Histone2B (direct cellular substrate of MST1) and β-cell apoptosis (cleaved Caspase 3 & PARP) were analyzed by western blotting. MST1 or dominant-negative MST1 were overexpressed by plasmid transfection into human islets and CM9 cells and its direct effect on β-cell apoptosis (TUNEL assay & caspase 3 activation) and function (GSIS, PDX1 localization) analyzed. MST1 activation was investigated in isolated islets from a patient with T2DM and from an animal model of T2DM, the high fat/ high sucrose diet fed mouse (HFD).
Results: MST1 cleavage & phosphorylation was increased in human islets and CM9 cells exposed to IL-1β/IFNγ mixture, H_2O_2 or increasing glucose concentrations (11.1-33.3 mM). This correlated with increased P-H2B, P-JNK and apoptosis. We found the JNK pathway as a mediator of MST1-induced apoptosis, because JNK inhibition (by JNK inhibitor SP600125) diminished caspase-mediated MST1 cleavage and apoptosis. In isolated islets from patients with T2DM as well as from HFD mice, MST1 activation was increased and correlated with JNK activation and apoptosis. Inhibition of endogenous MST1 activity by overexpression of dominant negative MST1 inhibited apoptosis induced by a diabetic milieu. In contrast, overexpression of MST1 increased β-cell apoptosis 4-fold (P>0,05) and reduced glucose stimulated insulin secretion 1.6-fold (p>0,05), indicating that MST1 alone is sufficient to promotes β-cell failure. By an in vitro kinase assay using recombinant MST1 and PDX-1 as substrate we found that MST1 phosphorylates PDX-1. In line with this MST1 overexpression induced PDX-1 shuttling from the nucleus to the cytosol, providing an explanation for the impaired insulin secretion.
Conclusion: Our results suggest that MST1 is a critical mediator of impaired β-cell function and apoptosis. Inhibiting the MST1-pathway could be an important strategy to prevent β-cell apoptosis.
Keywords: Mammalian sterile 20-like kinase 1 (MST1), apoptosis, diabetes, β-cell, c-Jun N-terminal kinase (JNK) and Pancreatic Duodenal Homeobox-1 (PDX-1).

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Islet human amylin oligomer formation is differentially correlated with beta cell death and diabetes onset between homozygous and hemizygous human amylin transgenic mice

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Background and aims: One of the pathological features of type-2 diabetes mellitus (T2DM) is the presence of islet amyloid deposits comprising mainly human amylin (hA)/hIAPP. Recent studies suggested that soluble oligomers of human amylin may be the primary cause of β-cell damage and thus contribute to the onset/development of T2DM. However, the molecular basis of this process remains to be fully elucidated. We aimed to investigate the connection between soluble oligomers and hA cytotoxicity, and their correlation with diabetes development using a rodent model of diabetes.
Materials and methods: We performed a comparative phenotypic analysis of homozygous and hemizygous hA transgenic mice for hA in their islet β-cells. Oligomer formation was studied by detection of amylin oligomer-like immunoreactive material (AOLIM) with specific anti-oligomer antibodies using immunofluorescence techniques. A co-localization study of oligomerization was carried out wherein islet cell hormones were detected by multi-labelled immunofluorescence. Apoptosis was assessed by active-caspase-3 staining.
Results: Both homozygous and hemizygous hA transgenic mice developed spontaneous diabetes associated with different elevations in hA levels and with different time frames of disease onset and death. Abnormal β-cell function and apoptosis were detected before early onset of hyperglycaemia in homozygous animals, while β-cell function (insulin secretion) remained normal till late diabetes onset in hemizygous mice. Interestingly, intracellular and extracellular AOLIM was clearly detectable before onset of diabetes in all transgenic animals with strong correlation with β-cell death in homozygous mice. However, there was no correlation between the appearance of AOLIM and the time of cell death occurring in hemizygous mice, implying a difference in the size and/or the extent of cytotoxic oligomerization in these animals. We also found that rapid β-cell depletion occurs soon after appearance of cytotoxic oligomers and onset of diabetes in homozygous animals whereas apoptosis developed slowly after the later onset of diabetes in hemizygous animals, which exhibited significant β-cell loss in a much later time frame.
Conclusion: The difference in time at which oligomerization occurs in relation to cell death and diabetes onset between homozygous and hemizygous hA-transgenic mice may suggest a dose-dependent cytotoxic effect of hA oligomers. The findings from this study will provide new insights on the cellular fate of oligomers and further enhance our understanding of amyloidosis and T2DM.
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Dual effect of advanced glycation end products in pancreatic islet apoptosis and the protective role of benfotiamine and MitoQ

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Background and aims: Loss of beta cell function hastens the deterioration of metabolic control in people with type 2 diabetes. Besides lipo- and glucotoxicity, advanced glycation end products (AGEs) seem to contribute to this process by promoting islet apoptosis. In other tissues, AGEs interact with their specific receptors (RAGEs) and elicit reactive oxygen species (ROS) generation and NF-kB activation. In order to investigate the temporal effect of AGEs on islet apoptosis as well as the potential of antioxidant compounds to decrease islet damage caused by AGEs,
Materials and methods: Rat pancreatic islets were treated for 24, 48, 72 and 96 h with either AGEs generated from co-incubation of bovine serum albumin (BSA) with D-glyceraldhyde (GAD, 5 mg/mL) or BSA (5 mg/mL, control). Apoptosis was evaluated by quantification of DNA fragmentation (ELISA), caspase-3 enzyme activity and detection of mitochondrial permeability transition (MitoProbe JC-1). The expression of the genes *Bax*, *Bcl2* and *Nfkb1* was evaluated by RT-qPCR. In the time points at which increased apoptosis was detected, the effect of two antioxidant compounds was evaluated: benfotiamine (350 μM), a liposoluble vitamin B1, and Mito Q (1 μM), a derivative of ubiquinone targeted to mitochondria
Results: In 24 and 48 h, AGEs elicited a significant decrease in the apoptosis rate in comparison to the control condition concomitantly with a significant increase in the RNA expression of the antiapoptotic gene *Bcl2* and a significant decrease in the *Nfkb1* RNA expression. In contrast, after 72 and 96 h, AGEs promoted a significant increase in the apoptosis rate in comparison to the control condition concomitantly with a significant decrease in *Bcl2* RNA expression and a significant increase in *Nfkb1* RNA expression. Benfotiamine and Mito Q were able to decrease the apoptosis rate of islets exposed to AGEs for 72 and 96 h.
Conclusion: AGEs exerted a dual effect in cultured pancreatic islets, being protective against apoptosis after short exposition but proapoptotic after prolonged exposition. Mito Q and benfotiamine deserve further evaluation as drugs that could offer islet protection in conditions of chronic hyperglycemia.
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Galectin-3 deficiency reduces immune-mediated beta cell destruction *in vitro*

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Background and aims: Galectin-3 (Gal-3), a multifunctional beta-galactoside binding lectin, affects numerous biological processes and is implicated in the pathogenesis of several inflammatory/autoimmune disorders. It has been shown that Gal-3 may increase inflammatory responses through its function in cell activation, cell migration, or inhibition of apoptosis. We have recently reported that Gal-3-deficient (Gal-3$^{-/-}$) mice are relatively resistant to diabetogenesis induced by multiple low doses of streptozotocin. Little is known however, about the relevance and influence of the endogenous Gal-3 at the level of target tissue (pancreatic islets). It is established that, in Type 1 as well as in Type 2 diabetes, beta-cell function and viability are progressively disturbed through a complex process of inflammation. In addition, hyperlipidemia and inflammation, when acting in concert, may carry out a powerful attack upon pancreatic beta cells. In this context, our aim was to analyze the possible role of endogenous Gal-3 in beta cell survival and death pathways following *in vitro* treatment with cytokines and one of the most common free fatty acid -palimitic acid.
Materials and methods: Pancreatic islets isolated from C57BL/6 mice or Gal-3-null mice were incubated with palmitate and/or concanavalin A-stimulated spleen-cell culture supernatant containing soluble immune cell products. Alternatively, the cooperative effect of culture supernatant components was mimicked with the combination of recombinant cytokines (IL-1β+TNF-α+IFN-γ). After incubation, beta cell apoptosis was determined by MTT test, or histone-DNA ELISA. Expression profiles of various related genes were determined at mRNA and protein levels by real time PCR, and Western blot, respectively.
Results: Targeted disruption of *Gal-3* gene attenuated palmitate-induced beta cell lipotoxicity. Moreover, Gal-3$^{-/-}$ pancreatic islets were more resistant than wild type counterparts to cytokine induced apoptosis. The transcription of anti-apoptotic Bcl-2 was up-regulated in Gal-3$^{-/-}$ islets only, whereas in wild type islets it was down-regulated. Although Bcl-xL mRNA was up-regulated in both groups of islets, the increase was more pronounced in Gal-3$^{-/-}$ islets. In contrast, the transcription of pro-apoptotic Bid was down-regulated in Gal-3$^{-/-}$ islets only. Interestingly, basal levels of anti-apoptotic proteins were higher in Gal-3$^{-/-}$ islets than in wild type islets, further suggesting favouring of cell protective over pro-apoptotic molecules in molecular regulation of Gal-3$^{-/-}$ beta cell survival.
Conclusion: Our findings clearly show that the absence of Gal-3 confers beta cell protection from the lethal insult of different agents, namely cytokines and saturated free fatty acids. This protection is probably mediated by differential regulation of pro- and anti-apoptotic molecules within the beta cells. The study thus provides *in vitro* evidence for a critical role of Gal-3 in destruction at the level of pancreatic islets and suggests a new therapeutic strategy for the treatment of diabetes based on the selective inhibition of Gal-3 activity.
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Ectopic overexpression of acute stress protein p8 reduces streptozotocin (STZ)-induced apoptosis in INS-1E (STZ) beta cells

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Background and aims: Protein p8 is associated with proliferation and tissue protection. p8 knock out animals display enhanced lethality after LPS treatment and enhanced pancreatitis-induced tissue damage in the exocrine pancreas. Within the endocrine pancreas own previous work characterised p8 as a glucose-dependent mediator of beta cell proliferation. Here we investigate the cell protective properties of p8 in response to STZ treatment in INS-1E beta cells.
Methods and results: Exposure of native INS-1E to STZ strongly upregulates activity of the apoptosis effector molecules caspases 3 and 7 (Chemiluminescence assay) resulting in cell death with a 24 h LD50 of 0.66 mM STZ (MTS). In parallel, 0.66 mM STZ acutely induces endogenous p8 gene expression (qPCR), which peaks after 6 h and fastly declines to vehicle control levels after 12 h. STZ-induced p8 gene expression (6 h) was further strictly dose-dependent up to 1 mM STZ and gets saturated at 3.3 mM. To investigate effects of high p8 levels on viability and apoptosis, we generated INS-1E beta cells with stable p8 overexpression under the control of a CMV promoter (p8-INS1) and empty plasmid control cells (mock-INS1). Under basal conditions, p8-INS1 cells as compared to mock controls demonstrate substantially enhanced viability resulting in significantly increased cell numbers after 5 days in culture. Also under tissue stress conditions, viability of p8-INS1 was substantially enhanced if exposed to 0.33 to 3.3 mM STZ for 24 h. These findings correspond to significantly lowered (about 60%) 0.33 to 1 mM STZ-induced activation of caspases 3 and 7 after 8 h.
Conclusion: These results demonstrate that, in beta cells, p8 is acutely induced by tissue stress and mediates enhanced viability and proliferation and reduces STZ-induced apoptotic cell death.
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Paracrine signaling loops in adult pancreatic islets: Netrins modulate beta cell apoptosis via Neogenin and Unc5a

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Background and aims: Adult pancreatic islets contain multiple cell types that produce and secrete well characterized hormones including insulin, glucagon and somatostatin. Although it is becoming increasingly apparent that islets release and respond to more secreted factors than previously thought, systematic analyses are lacking. The aims of the present study were to identify potential autocrine/paracrine islet growth factor loops and to characterize the function of a family of previously unreported islet secreted factors and their receptors.
Materials and methods: Gene expression databases, islet specific SAGE and Tag-Seq libraries, and microarray datasets of FACS purified human beta-cells were used to compile a list of secreted factors and secreted factor receptors present in mouse or human islets. The presence of Netrins and their cognate receptors were assessed using RT-PCR, western blot analysis, and immnofluorescence staining. The roles of Netrin-1 and Netrin-4 in beta-cell function, apoptosis, and proliferation were also examined.
Results: A list of 230 secreted factors and 238 secreted factor receptors (189 factor-receptor pairs) were found in islets. This genome-wide analysis led us to characterize the role of netrins in adult pancreatic beta-cell apoptosis. The presence of netrins and their cognate receptors (Neogenin, DCC, and Unc5A-D) were confirmed using RT-PCR, western blot analysis, and immnofluorescence staining. Down-regulation of caspase-3 activation was observed when MIN6 cells were exposed to exogenous Netrin-1 and Netrin-4 under hyperglycaemic conditions, and the observed effects were not dependent on induction of insulin secretion. Reduction in caspase-3 cleavage was linked to the decrease in dependence receptors, Neogenin and Unc5A.
Conclusion: Together, our results highlight the large number of potential islet growth factors and point to a context-dependent pro-survival role for netrins in the adult pancreatic beta-cells. Since diabetes results from a deficiency in functional beta-cell mass, these studies are an important step towards developing novel therapies to improve beta-cell survival.
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The fibrosis in pancreatic islets at an early stage of life involves in diabetes development in obese diabetic db/db mice

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Background and aims: The *db/db* mice develop diabetes with severe insulin resistance and limited capacity of insulin secretion. On the other hand, the

ob/ob mice with a similar genetic background do not present diabetic manifestation because of a compensatory hypersecretion of insulin. We previously reported that the *ob* gene, but not *db* gene, homozygote acquires a compensatory mechanism of beta cell protection probably through anti-oxidative stress mechanism at 12 weeks of age. In this study, to further investigate the molecular mechanism of diabetes development in *db/db* mice, the beta cell function and gene expression profiles specific for pancreatic islets in *db/db* mice were compared with those in *ob/ob* mice at an early stage of life, when metabolic parameters were not different between two strains of mice.
Materials and methods: Body weight (BW), fasted blood glucose (FBG), fasted insulin (FIRI), TG and FFA in *db/db*, *ob/ob* and lean littermates *m/m* mice were measured at 6 weeks of age. The beta cell mass and cell ratio were assessed by histological analysis of the islet tissue. In the first step of molecular analysis, we examined comprehensive gene expression profiles of isolated islets by the cDNA microarray analysis. Furthermore, gene expressions specific for the core area of pancreatic islet were analyzed by Laser Capture Microdissection (LCM) method and real time RT-PCR. Primer pairs encoding genes associated with pancreatic hormones, cell proliferation, apoptosis, cell cycle, and oxidative stress were prepared, and real-time RT-PCR with Sybr Green was applied at 6 week old mice.
Results: BW in *db/db* mice was significantly greater than that in *m/m* mice, but was lower than *ob/ob* mice. FBG and FIRI in *db/db* and *ob/ob* mice were significantly higher than those in *m/m* mice, but no difference was observed between *db/db* and *ob/ob* mice (FBG: 93.1±6.0 in *db/db*, 89.9±5.8 in *ob/ob*, 53.6±3.6 mg/dl in *m/m*, FIRI: 1.84±0.10, 1.58±0.12, 0.12±0.01 ng/ml at 6 weeks of age, $p<0.05$, respectively). TG and FFA levels in *db/db* and *ob/ob* mice were significantly higher than those in *m/m* mice. The beta cell mass and cell ratio in *db/db* was significantly less than those in *ob/ob* and *m/m* mice at 6 weeks of age (cell mass: 1.03±0.04 in *db/db*, 1.16±0.02 in *ob/ob*, 1.12±0.06 mg in *m/m*, and cell ratio: 81.1±0.1, 83.0±0.5, 82.9±0.4%, respectively). The cDNA microarray analysis at 6 weeks of age demonstrated no significant difference in the islet gene expressions related with cell differentiation/proliferation and ER/oxidative stress among three groups. On the other hand, the tissue fibrosis and inflammation related genes were highly expressed in *db/db* mice than in *ob/ob* mice. The LCM and real-time PCR analysis revealed a significant increase in insulinIIgene expression in both *db/db* and *ob/ob* mice. Nkx6.1 gene related with cell differentiation was significantly increased in *ob/ob* compared with *db/db* and *m/m* mice. The cell proliferation, cell apoptosis and ER/oxidative related gene expressions were not different among three groups of mice. On the other hand, fibronectin and collagen type 1 gene expressions were significantly up-regulated in *db/db* mice compared with *ob/ob* and *m/m* mice.
Conclusion: The present results suggest that the preceding tissue fibrosis in pancreatic islets at an early stage of life may be involved in diabetes development in *db/db* mice.

PS 32 Beta cells under stress

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A lipidomic screen of lipotoxic pancreatic beta cells reveals links between ceramide accumulation in the endoplasmic reticulum (ER), impaired protein trafficking, ER stress and apoptosis

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Background and aims: An intrinsic effect of saturated fatty acids (FAs) on the pancreatic β-cell contributes to the β-cell death that occurs in Type 2 diabetes. The exact mechanism by which saturated FAs bring this about remains elusive, but is mediated, in part, by endoplasmic reticulum (ER) stress. This study attempts to pinpoint the toxic lipid metabolite responsible for ER stress and apoptosis and offer mechanistic insight into its action.
Materials and methods: Pancreatic β-cell line, MIN6, and their palmitate-resistant (PR) negative controls, were treated chronically (48 h) with 0.4mM palmitate:0.92% BSA, as a model of lipid oversupply. A comprehensive lipidomic screen of β-cells, was undertaken via mass spectrometry. The level of apoptosis (DNA fragmentation ELISA) was determined following palmitate treatment and in combination with genetic intervention via overexpression of glucosylceramide synthase. Western blotting to measure ER stress-induced protein, CHOP, was performed and ER-to-golgi trafficking using a VSV-G_{t045}-GFP reporter assay were measured. Subcellular fractionation was undertaken using density gradient centrifugation.
Results: Palmitate pretreatment caused only very modest increases in mass of some major neutral phospholipids (triglyceride and phosphatidylcholine). There was also a 70% increase in glycosylceramide that was manifest in most side-chain species and cellular locations. Although total ceramide levels where unchanged, there were specific increases in the ER (56%) and lysosomal compartments (23%). A selective decrease in sphingomyelin species with side chain lengths greater than 20 carbons in length was also observed, consistent with a defective vesicular trafficking of longer chain ceramide species that impacts on corresponding sphingomyelin forms. Protein trafficking was also reduced by palmitate pretreatment, and this was overcome by overexpression of glucosylceramide synthase which metabolises ceramide. ER stress and apoptosis was also reduced under these conditions. The protein chaperone, phenyl-butryric acid did not rescue the sphingolipid alterations due to palmitate, nor were they reproduced by thapsigargin or tunicamycin, showing that the alterations are upstream, not downstream, of ER stress.
Conclusion: Our data provides a mechanistic link between ceramide accumulation and ER stress in lipotoxic β-cells and thereby reconciles two previously disparate areas of prior investigation. Specifically, we demonstrate that increases in ER ceramide underlie apoptosis in even mild models of lipotoxicity. Our results also lend support to the idea that protein overload, secondary to reductions in ER-to-golgi protein trafficking caused by ceramide, contributes to the induction of ER stress.
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The decrease in insulin secretion by prednisolone involves activation of the endoplasmic reticulum stress pathway in INS-1E cells

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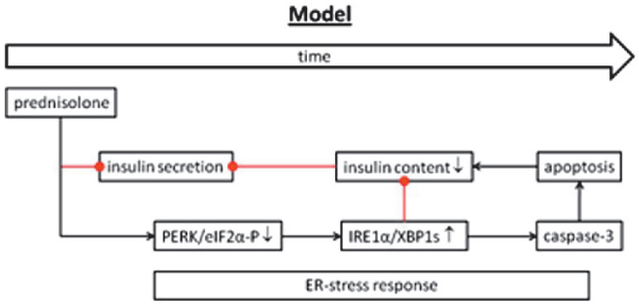
Background and aims: The glucocorticoid prednisolone (PRED) impairs multiple aspects of beta cell function in man, resulting in a decreased insulin secretory potential. One of the possible underlying mechanisms could be induction of endoplasmic reticulum (ER) stress, which leads to inhibition of gene transcription and protein synthesis. Here, we investigated whether PRED-induced ER stress contributes to beta cell dysfunction in INS-1E cells.
Materials and methods: INS-1E cells were treated with 700nM PRED, and insulin secretion in response to glucose and KCl was determined at different time points. mRNA and protein expression of various ER stress markers

(PERK, eIF2α, IRE1α, and XBP1s) were determined by real-time PCR and western blot, respectively.
Results: Exposure of INS-1E cells to PRED caused a time-dependent reduction in glucose-stimulated insulin secretion (GSIS). Inhibition of GSIS was already present 1 h after the addition of PRED, and occurred without affecting insulin content. This short-term treatment had no inhibitory effect on KCl-stimulated insulin secretion (KSIS). By contrast, prolonged incubation with PRED (20 h) abrogated both GSIS and KSIS, lowered insulin and PDX1 mRNA and protein expression, and increased the expression of the apoptosis marker cleaved caspase 3. These effects of PRED were preceded by a decrease in the phosphorylation of PERK and its substrate eIF2α, and an increase in the expression of the endonuclease IRE1α, and its target spliced XBP1s. Finally, all PRED-induced effects were reversed by the glucocorticoid receptor antagonist RU486.
Conclusion: PRED acutely inhibits insulin secretion by a mechanism which does not involve alteration of insulin content. By contrast, inhibition of insulin secretion by chronic PRED exposure can be ascribed to reduced insulin content resulting from ER-stress. We propose that PRED-induced dephosphorylation of the PERK/eIF2α-pathway lowers PDX1- and insulin- mRNA and protein levels through activation of IRE1α/XBP1s-pathway.



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Losartan protect human pancreatic islets from glucotoxic ER and oxidative stress

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Background and aims: In animal models and humans, chronic hyperglycemia is associated with alterations in beta-cell mass and function. Chronic high glucose concentrations increased glucose metabolism through oxidative phosphorylation. This causes mitochondrial dysfunction and excess production of reactive oxygen species (ROS) in beta-cells due to their low levels of ROS-detoxifying enzymes. Beta-cells present a developed ER in order to answer to high demand for synthesis of insulin. Recent data suggest that ER stress is present in human beta-cells and that this could be a common mechanism for the two major pathophysiological events in type 2 diabetes, insulin resistance and beta-cell failure. Data from prospective studies suggest a significant reduction in the risk of type 2 diabetes after blockade of the renin angiotensin system (RAS). Since RAS has been found in pancreatic islets, we hypothesized that these beneficial effects could be attributed to direct actions on islets. Our present study evaluates the effects of the Angiotensin II receptor blocker Losartan on stress induced by glucose in isolated human beta cells.
Material and methods: Human islets from 6 distinct donors were studied following 96 hrs in culture in presence of 5.5 mmol/l or 16.7 mmol/l glucose concentrations with or without 5 µmol/l Losartan during the last 48 hrs. ER stress-related mRNA, INS and VEGF mRNA expressions were detected by real-time RT-PCR, GRP78 protein expression and eIF2 α phosphorylation by Western-blot. Angiogenesis-related protein expression was measured by protein arrays. ROS levels were determined by measuring DCF oxidation and insulin secretion by IRMA.
Results: Insulin gene expression and protein secretion were significantly increased by 6.5 times at 16.7 mmol/l glucose compared to 5.5 mmol/l glucose (p=0.03; p=0.006, respectively). This increase was not modified by Losartan. Chronic high glucose exposure decreases VEGF mRNA and protein expression levels (20% and 64%, p=0.05 and p=3.10-6, respectively). Protein arrays showed that high glucose reduced expression of additional factors such as IGFBP2 by 53% (p=5.10-7) and IL8 by 24% (p=0.008). Losartan was able to reverse effects observed on protein expression of VEGF by 25%, p=0.05, IGFBP-2 by 100%, p=0.002 and IL8 by 100%, p=0.02. Chronic high glucose up regulates ROS levels by 60% (p=0.02). This effect was half abrogated by Losartan. GRP78, spliced XBP-1 mRNA expressions increased with glucose concentration, (x2.2;p<0.012, x1.5 ;p<0.009, respectively). The expression of CHOP, an ER stress marker of apoptosis, remained the same, suggesting that this chronic glucose treatment caused mild ER stress but not cell death. Moderate increase of GRP78 protein expression (x1.4) and eIF2α phosphorylation (x1.3) was observed with high glucose concentration. Addition of Losartan to 16.5mmol/l glucose medium reduced significantly ER stress markers' expression, GRP78 mRNA by 55% (p< 0.01), XBP1-s mRNA by 51% ;p<0.02) as compared to high glucose condition alone.
Conclusion: Chronic high glucose exposure of human islets reduces angiogenesis and parallely increases oxidative and ER stress. Blockade of islet RAS by Losartan modulates angiogenesis and protects islet cells against ER and oxydative stress. These findings may have important therapeutic consequences both for prevention of type 2 diabetes and islet transplantation.
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New players in the beta cell ER stress response: UFM1 and UFBP1

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Background and aims: To fulfil their demanding metabolic task - the regulated synthesis, storage and secretion of insulin - pancreatic beta cells possess a highly developed endoplasmic reticulum (ER). This may explain why beta cells are vulnerable for ER overload as was illustrated by heterozygous eIF2alpha^{ser51ala} mutant mice fed a high fat diet. Therefore, optimal functioning of the ER is essential for proper insulin folding and beta cell survival. Chronic imbalance between protein synthetic demands and ER folding capacity leads to ER stress and activation of the ER stress response to restore ER homeostasis. The aim of the present study was to identify new players in the ER stress response, starting from genome wide mRNA expression analysis and genes with unknown function which are highly expressed in islets. Ubiquitin-fold modifier 1 (UFM1), the most recently identified ubiquitin-like protein with unknown function, is proposed as being a new player in the beta cell ER stress response.
Materials and methods: mRNA expression analysis was performed by Affymetrix microarrays and confirmed by RT-PCR. Using STrEP-tag affinity purification and mass spectrometry we identified a new target of UFM1. The cellular localisation of UFM1 and its target was identified via cellular fractionation and immunocytochemistry. The effect of free fatty acids and of cyclopiazonic acid on ER stress and on apoptosis was analysed in INS1 cells treated with *Ufm1* specific siRNA. The influence of UFM1 and its target on ER associated protein degradation was analysed by overexpression of CD3δ, a known target of ERAD, in siRNA treated INS1 cells.
Results: Among a panel of 20 investigated organs and tissues, UFM1 is highly expressed in pancreatic islets of Langerhans and in other protein-secreting exocrine cell types (pancreatic acini, salivary glands, and seminal vesicles), both at the mRNA and protein level. We identified a highly conserved, unknown protein as a target of UFM1 and named it UFBP1 for UFM1-binding protein 1 containing a PCI domain. Both UFM1 and UFBP1 co-localised in the ER. Pharmacological induction of ER stress by cyclopiazonic acid (25 µM) enhanced expression of both *Ufm1* and *Ufbp1* (respectively 3.7-fold and 5.3-fold) in INS1 cells. This pharmacolocial effect was partially mimicked by addition of the free fatty acids oleate and palmitate which enhanced *Ufbp1* expression (respectively 1.7-fold and 2.4-fold). siRNA-mediated reduction of *Ufm1* or *Ufbp1* expression enhanced apoptosis upon ER stress stimulation and diminished ERAD as evidenced by less degradation of CD3δ.
Conclusion: Our data show for the first time that UFM1 via its target protein UFBP1 is a new molecular player in ERAD and ER stress-induced beta cell apoptosis.
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Cell type-specific transcriptional regulation of 4E-BP1 under ER stress in MIN6 beta cells

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Background and aims: Recent studies reveal that translational control of protein expression is activated in cells under stress conditions. We have recently identified translational control involving a transcriptional suppressor 4E-BP1 is important for beta-cell survival under ER stress and oxidative stress conditions. The *Eif4ebp1* gene, encoding 4E-BP1, is also shown to be a direct target of a transcription factor ATF4, a master regulator of gene expression in stress responses, in MIN6 insulinoma cells. In the current study, we investigated 4E-BP1 expression under ER stress in various cell lines.
Materials and methods: MIN6 cells and other cell lines were treated with thapsigargin and subjected to Western blot analysis. Luciferase reporter constructs containing varying lengths of the *Eif4ebp1* gene were generated and introduced into MIN6 cells and mouse embryonic fibroblasts (MEFs). Transcriptional activity of the *Eif4ebp1* gene fragments were analyzed in these cells.
Results: Although ER stress increased mRNA and protein levels of 4E-BP1 in MIN6 cells, little increases in 4E-BP1 protein levels were observed in response to thapsigargin in MEFs, COS7 and NIH3T3 cells as well as eight other cell lines tested, despite marked increases in stress marker proteins, ATF4 and CHOP. Expression of 4E-BP1 mRNA levels were not significantly increased in MEFs treated with thapsigargin neither, suggesting that lack of 4E-BP1 induction occurred at the transcriptional level. In order to gain insight into the difference in 4E-BP1 expression between MIN6 cells and MEFs, we examined different portions of the *Eif4ebp1* promoter and enhancer. An approximately 1 kbp region of the *Eif4ebp1* gene was found to be attributable to the different expression in response to thapsigargin treatment. Furthermore, we found that Smad signaling pathway are different between MIN6 cells and MEFs, and that lack of expression of a transcriptional coactivator of Smad might be involved in different expression of 4E-BP1 under ER stress.
Conclusion: Our results provide the evidence for cell type-specific transcriptional regulation of 4E-BP1 in MIN6 cells under stress conditions.
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Metallothioneins and beta cell function during glucose or palmitate stress

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Background and aims: Metallothioneins (MT) are metalloregulatory proteins partly controlling intracellular and extracellular zinc metabolism. MT I and II are low molecular weight (6-7 kDa) nonenzymatic cysteine-rich proteins. Both are important for the regulation of pathophysiological processes that depend on zinc and the processes during which oxidative stress mobilizes zinc. MTs may influence survival of cells in several organs and affect transmitter systems depending on zinc ions for optimal function. MTs are highly inducible by e.g. endotoxins and glucocorticoids as well as by heavy metals such as zinc or copper. Beta cells depend on zinc ions for storage and secretion of insulin. Zinc metabolism in beta cells is regulated by a number of proteins transporting zinc across membranes, notably ZnT8 and ZnT3. Furthermore, cell survival may be influenced by zinc levels and thus by MTs. The aim of this study was to investigate the effects of MTs on insulin secretion, gene expression of the apoptosis-regulating genes BAX and BCL2 as well as the expression of ZnT3 and ZnT8.
Materials and methods: Glucose sensitive INS-1E cells were examined after exposure to varying doses of glucose or palmitate for 24 hours. 1. Beta-cells were exposed to 3.3mM, 6.6mM or 21mM glucose with or without Zn_7Metallothionein-2A (rabbit). 2. Beta-cells were exposed to palmitate 0 mM, 0.4 mM or 1.0 mM with or without Zn_7Metallothionein-2A (rabbit). All experiments were performed in replicas of 6. Gene expressions of BAX, BCL-2, Insulin, Zinc-transporters (ZnT3, ZnT5, ZnT8) and Metallothioneins (1A and 3) were investigated by RT-PCR and insulin secretion and content were determined by ELISA.
Results: Zn_7Metallothionein-2A did not change insulin gene expression. During basal conditions MT decreased insulin secretion (6.6 mM glucose) but MT increased the insulin secretion during stress (high glucose or high palmitate). The ZnT8 gene expression was decreased by Zn_7Metallothionein-2A at high palmitate and glucose concentrations. The ZnT3 gene expression was increased by Zn_7Metallothionein-2A after palmitate but decreased by MT at basal conditions. BAX/BCL ratio was decreased by Zn_7Metallothionein-2A during glucose stress, but showed no change with palmitate stress.
Conclusion: Manipulating intracellular zinc metabolism by exogenous MTs influence insulin secretion, the expression of zinc transporting proteins and genes related to apoptosis in a complex manner. Exogenous MTs seem to favour an enhanced insulin secretion during glucose or lipid stress and may affect the expression of apoptotic genes in a favourable direction. Induction of MTs may favourably influence beta cell function and survival, a complex mechanism of action involving also ZnTs needs clarification.
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Dysfunction and proliferation of pancreatic beta cells by cyclic intermittent hypoxia

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Background and aims: Sleep apnea syndrome (SAS) is a highly prevalent sleep disorder characterized by cyclic intermittent hypoxia (IH). Accumulating evidence suggests that SAS is associated with glucose intolerance and insulin resistance independent of age, gender, smoking status, body mass index, and waist circumference. In addition to the development of glucose intolerance and insulin resistance, the progression to type 2 diabetes is dependent on the impairment of glucose-induced insulin secretion (GIS) from pancreatic beta cells and the compensatory replication of pancreatic beta cells to combat the presence of insulin resistance. However the direct effects of IH on GIS and beta cell replication have been obscured.
Materials and methods: Hamster insulinoma HIT-T15 cells, rat insulinoma RINm5Fcells, and isolated rat islets were exposed either to sustained hypoxia (SH) (1% O_2), 64 cycles/24 hours of intermittent hypoxia (IH) (5 min hypoxia/10 min normoxia (21%O_2)), or normoxia for 24 hours. After the treatment, HIT-T15 cells and rat islets were incubated in RPMI1640 medium containing 5.5 mM glucose (LG), or 22 mM glucose (HG) for 1h in normoxia. Insulin in the medium was measured by an ELISA kit. Real-time RT-PCR of insulin, CD38, glucose transporter 2, glucokinase, sulfonylurea receptor1, and L-type Ca channel1.2 was performed using normoxia- or IH-treated islet RNA as template. Cellular proliferation and apoptosis were measured by WST-8 assay and TUNEL method, respectively.
Results: The GIS of IH-treated HIT-T15 cells was attenuated, whereas GIS of the cells treated with normoxia was increased by HG ($p<0.01$). GIS from the isolated rat islets was also abolished by the treatment of IH. Real-time RT-PCR revealed that the level of insulin mRNA was unchanged by IH treatment. We then examined the mRNA levels of several genes involved in GIS in the islets. The mRNA levels of glucose transporter 2, glucokinase, sulfonylurea receptor1, and L-type Ca channel1.2 in IH-treated-islets were similar to those in normoxia-treated islets. In contrast, the mRNA level of CD38 in IH-treated islets was significantly lower than that in normoxia-treated islets (39% of the control), indicating possible dysfunction of the CD38-cyclic ADP-ribose signal system for insulin secretion in IH-treated beta cells. By WST-8 assay, the HIT-T15 cell proliferation was significantly increased by IH ($p<0.01$) and decreased by SH ($p<0.01$) compared to that of normoxia-trated cells. The cellular proliferation of RINm5F cells was also increase by IH, whereas apoptosis in RINm5F cells and HIT-T15 cells were unchanged by IH treatment. The mRNA level of Reg I, an autocrine/paracrine beta cell growth factor, was significantly increased by IH-treated RINm5F cells.
Conclusion: These results indicate that IH stress attenuates GIS and stimulates beta cell proliferation as compensatory response. It is quite possible that the cyclic change of hypoxia-reoxygenation, which occurs in SAS patients, induces beta cell dysfunction and proliferation, resulting in glucose intolerance and type 2 diabetes.

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Pancreatic beta cell Uchl1 regulates glucose homeostasis in high-fat fed mice

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Pancreatic beta-cells are professional secretory cells with substantial protein production and degradation demands. Defects in the production or secretion of insulin contribute to the pathogenesis of diabetes. Pancreatic beta-cells express ubiquitin C-terminal hydrolase L1 (Uchl1) is a component of the ubiquitination pathway, which deubiquitinizes polyubiquitin into monomers after proteasomal degradation, but its functional role has not been studied. Our previous proteomic screen suggested that Uchl1 protein was increased by the fatty acid palmitate at 5 mM glucose. Here, western blot analysis confirmed that Uchl1 protein was increased by 24-hour palmitate treatment at 5 mM, but surprisingly not 25 mM, glucose. Ubiquitin levels were reduced at high glucose condition independent of palmitate treatment. Mice harbouring the gracile axonal dystrophy (gad) mutation in the Uchl1 gene were used to study the role of Uchl1 in lipid-induced pancreatic beta-cell dysfunction. Gad mice exhibited normal body weight, glucose tolerance, and insulin tolerance, beta-cell mass and islet architecture on a normal chow diet. However, after 4 weeks of high fat feeding, male gad mice became glucose intolerant and without changes in insulin sensitivity. In these gad mice, the first phase of insulin secretion was impaired in both in vivo and in vitro studies. Intracellular calcium level was not affected, suggesting a specific defect in the exocytosis of insulin. Islets from Uchl1 mutant mice also exhibited an increase in ER-stress and apoptosis. Together, these data suggest that beta-cell UCHL1/gad may play a previously unappreciated role in glucose homeostasis.
Supported by: CDA

PS 33 Micro RNAs methylation and beta cell transcription

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Rapid alternations in DNA methylation patterns in insulin-producing beta cells correlate with changes in ambient glucose levels

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Background and aims: Elevated levels of glucose are characteristic of individuals with type 2 diabetes mellitus. Prolonged hyperglycemia is detrimental for insulin-producing beta-cells and leads to impaired glucose-stimulated insulin secretion and apoptosis. The harmful effects have been connected with changes in transcript levels of several genes. The aim was to determine whether extended episodes of hyperglycemia and the following alternations in gene expression are connected with changes in epigenetic patterns and, if so, such patterns can be reversed following a period of normoglycemia.
Materials and methods: Insulin-secreting INS-1E cells were cultured at 16.7 mM glucose for 1, 3, 5 or 7 days. For cells cultured for 7 days, the glucose concentration was reduced from 16.7 to 11 mM glucose during the last 2 days. Cells cultured at 11 mM glucose were used as controls. Cells were harvested after 1, 3, 5 and 7 days and total DNA extracted for analysis of DNA methylation. After sodium bisulfite treatment, DNA was amplified by PCR and subsequently sequenced. DNA methylation patterns were measured at four different sites in the repetitive DNA segment Long Interspersed Nucleotide Element (LINE) 1. The methylation status of this segment is known to be representative of global DNA methylation patterns.
Results: INS-1E cells cultured at 16.7 mM glucose for 24 hours showed no changes in methylation of the CpG sites within LINE1. In contrast, after 3 days a significant change in the methylation patterns was observed in cells exposed to 16.7 mM glucose. No further change was observed after 5 days of exposure to the hyperglycemic milieu. Interestingly, methylation patterns were reversed back to those observed in control cells when cells cultured at 16.7 mM glucose for 5 days were exposed to 11 mM glucose for an additional 2 days.
Conclusion: Exposure to elevated glucose levels induces global epigenetic changes in the beta-cell within only a few days. These changes can be reversed by normalization of the glucose levels. Glucose-induced epigenetic changes may thus contribute to altered transcription levels associated with hyperglycemia. These findings call for experimentation addressing epigenetic changes of specific genes. The results illustrate how environmental factors affect gene regulation and may give insight into mechanisms connected with development and reversal of hyperglycemia and thus serve as a model for disease progression and recovery in type 2 diabetes mellitus.
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A novel bioinformatics approach combining array profiling and independent component analysis identifying microRNAs with potential roles in beta cell maturation and dysfunction

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Background and aims: The selective β-cell destruction taking place in type 1 diabetes (T1D) is believed to involve a sensitivity to interleukin-1β (IL-1β) that is acquired during β-cell maturation and has been associated with the transcription factor Pancreatic and duodenal homeobox 1 (Pdx1). microRNA (miRNA) repress gene expression posttranscriptionally through mRNA degradation or translational inhibition. Several miRNAs have been associated with β-cell differentiation or dysfunction. The aim of the study was to identify candidate miRNAs by using a cellular model of acquired IL-1β sensitivity and a novel bioinformatics approach.
Materials and methods: Array profiling was performed on miRNA and mRNA expression levels in a β-cell line with inducible Pdx1 expression (INSrαβ) in response to Pdx1 induction alone or in combination with IL-1β exposure. Independent component analysis (ICA) was applied to decompose the mRNA profiling data into independent components (ICs), thus identifying biologically coherent groups of genes. For identification of inverse cor-

related miRNA and mRNA expressions Pearson correlation coefficients were calculated. Predicted miRNA targets were defined as mRNAs with a given miRNA 6mer seed match in their 3'UTR. INS-1 cells were used to verify the expression of the identified miRNAs.
Results: Array profiling identified eight miRNAs (miR-124, miR-128, miR-192, miR-194, miR-204, miR-375, miR-672 and miR-708) with differential expression in response to Pdx1 induction and/or IL-1β exposure (Bonferroni corrected $p<0.1$). Four of the eight miRNAs show a significant enrichment of predicted targets among highly inverse correlated mRNAs ($q<0.01$), indicating miRNA-mediated repression of these mRNAs. Performing ICA on the mRNA data revealed five highly significant ICs (Bonferroni corrected $p<0.0001$) correlating to the experimental conditions, as shown by a significant enrichment of known Pdx1 and IL-1β regulated genes in the respective ICs ($p<0.05$). Interestingly, all eight miRNAs can be represented (percent variance explained >97%) by a superposition of the five ICs. Further, testing for enrichment of ICA-identified genes in KEGG-annotated pathways identified 25 pathways e.g. Type 1 Diabetes Mellitus, Maturity Onset Diabetes of the Young and Type 2 Diabetes Mellitus ($q<0.05$). Except for miR-124, there was a good resemblance between the IL-1β-induced miRNA expression changes in the INS-1 cells and the Pdx1-induced INSrαβ cells.
Conclusion: The mRNA expression is influenced by a number of factors such as accessibility, transcription factor binding and miRNA repression, thus few mRNAs have a clear inverse correlation with their targeting miRNAs. Here, ICA was applied as an attempt to filter all the various contributions from each other. The result was an identification of eight miRNAs with potential involvement in β-cell maturation and dysfunction. Interestingly, the miRNAs appear to directly or indirectly affect pathways of relevance to disease mechanisms in T1D.

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MicroRNA miR-187 is reduced in human pancreatic islets from type 2 diabetic donors and in glucagon-secreting cells following palmitate treatment

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Background and aims: The pancreatic islet damage during type 2 diabetes (DM2) is mediated by lipotoxicity phenomena with consequent beta cell dysfunction and increase in glucagon secretion. *In vitro* treatment with palmitate is sufficient to mimic a functional damage in pancreatic islets similarly to what observed in DM2. MicroRNAs are small endogenous RNAs, whose function is to pair mRNAs 3'UTRs regions of protein-coding genes, negatively affecting their translation or stability. MicroRNAs are involved in cellular differentiation and proliferation as well as in endocrine pancreas development, in regulation of insulin secretion and of insulin signalling. We have previously shown a differential miRNAs expression profiling in DM2 vs control human pancreatic islets characterized by a major downregulation of miR-187 expression, which resulted virtually undetectable in DM2 islets. Consequently, the aim of the present study was to analyze the effects of palmitate treatment on the alpha cell component of the pancreatic islets in terms of miR-187 expression, in order to establish whether such treatment could mimic in vitro the scenario present in the type 2 diabetic islet.
Materials and methods: Experiments were performed employing the alphaTC1-6 cell line, which was treated with palmitate 0.5 mM for 48h. Quantitative analysis of miR-187 and of islet specific miR-375 and miR-7 expression was performed using stem loop specific primers for reverse transcription followed by real time PCR. All values were normalized to U6 endogenous RNA.
Results: A 49±22% reduction of miR-187 ($p<0.05$) was observed in alphaTC1-6 cells following palmitate treatment, while islet-specific miR-375 and miR-7, whose expression was not found altered in DM2 islets, resulted unaffected. Of note, the computational analysis of miR-187 mRNAs targets with TARGETSCAN and MIRANDA algorithms revealed genes of potential interest such as glucagon, the alpha-cell transcription factor Pax6 and the insulin receptor.
Conclusion: This study demonstrates that *in vitro* treatment with palmitate of alphaTC1-6 cells determines a reduction in miR-187 expression that is similar to that observed in pancreatic islets from type 2 diabetic patients, thus suggesting that an altered expression of miR-187 could be involved in the islet dysfunction in DM2 and therefore may represents a potential therapeutic target.
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MicroRNA differences in human islets and glucose-sensitive tissues

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Background and aims: We aim to identify microRNAs (miRNAs) that are expressed in the human pancreatic islets compared to other glucose-sensitive tissue such as liver and skeletal muscle. The hypothesis is that specific miRNAs expression affects the translation of messenger RNAs that are involved in glucose-stimulated insulin secretion (GSIS).
Materials and methods: We profiled 322 miRNAs in the islets of a human donor, as well as commercial liver and skeletal muscle RNA using locked nucleic acid (LNA)-based microarray. The eight most highly expressed miRNAs in islets compared to liver and skeletal muscle were selected, and expression in 16 additional human islet donors were validated using quantitative PCR. The effects of GSIS on miRNAs expression were investigated by incubating the islets for 1 hour at different glucose concentrations. Insulin release from intact islets was determined by radioimmunoassay. Bioinformatics predictions of miRNAs targets were filtered by available mRNA expression data, and enriched for gene ontology terms.
Results: We found a correlation between mir-375, mir-127 and mir-184 and insulin biosynthesis (Spearman R = 0,8022, p = 0,0005). Surprisingly, all three miRNAs were negatively correlated to insulin secretion. Hierarchical clustering of the global islet miRNAs profile allowed to separate islet-specific miRNAs from liver and muscle-specific miRNAs. Ten qPCR-validated miRNAs displayed expression differences in all tissues. Bioinformatic analysis revealed that the predicted miRNAs targets were enriched for genes with known role in insulin secretion.
Conclusion: In conclusion, high levels of islet mir-375, mir-127 and mir-184 may directly or indirectly affect insulin-secretion negatively. Our results also depict a role of islet-specific miRNAs in pathways of importance for type 2 diabetes and maturity onset diabetes of the young (MODY).
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Glucagon stimulates glucagon gene transcription in mouse and human islets by an autocrine feedback loop

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Background and aims: The glucagon gene is expressed in pancreatic alpha-cells, intestinal L-cells and specific neurons, but only the alpha-cell produces and secretes glucagon due to different posttranslational processing. We have demonstrated that secreted glucagon positively regulates the transcription of its own gene in the pancreatic alpha-cell line alphaTC1-9. The molecular mechanism underlying this positive autocrine feedback involves the glucagon receptor, protein kinase A and C and the transcription factor CREB. Because these observations were made utilizing a cell line system, the aim of this study was to verify whether this positive feedback mechanism also exists in mouse and human pancreatic islets.
Materials and methods: Mouse islets were isolated from 4 month old BALB/C mice and cultured for 72 h in RPMI 1640 medium containing 10% fetal calf serum (FCS). Human islets were cultured in RPMI 1640 medium containing 10% FCS for 72 h after arrival prior to experiments. Islets were stimulated by either 2 mM glucose for 15 min or 200 nM glucagon for 5 min at 11 mM glucose in RPMI 1640 medium containing 10% FCS. After stimulation the islets were returned to fully supplemented RPMI 1640 medium. For inhibitor experiments the islets were incubated with the pharmacological inhibitors for 30 min prior to and during the stimulation. 60 min after the start of stimulation RNA was isolated from the islets. Changes in glucagon mRNA levels were then determined by QPCR normalized to cyclophilinA mRNA levels.
Results: Stimulation of islets with low glucose (2mM) led to a 1.4±0.1 fold increase in glucagon mRNA levels. Addition of exogenous glucagon for 5 min resulted in a 3.0±0,1 (mouse) or 2.5±0.2 (human) fold increase of glucagon mRNA. This increase was abolished in both mouse and human islets by treatment with 20 nM glucagon receptor antagonist II (Merck), a specific pharmacological inhibitor of the glucagon receptor, demonstrating the critical importance of glucagon receptor signalling. To verify the involvement of protein kinase C and protein kinase A in the stimulation of glucagon gene transcription by glucagon, we incubated islets with either a protein kinase A inhibitor (100 μM RPCAMPS) or a protein kinase C inhibitor (150 nM bisindolylamide II). Either treatment abolished the stimulatory effect of glucagon in human and mouse islets.
Conclusion: Our findings demonstrate that secreted glucagon up-regulates glucagon gene transcription in both mouse and human islets through an au-

tocrine feedback loop involving the glucagon receptor and protein kinases A and C.
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Impact of a stabilized human reg3a protein on islet neogenesis and glycaemic control
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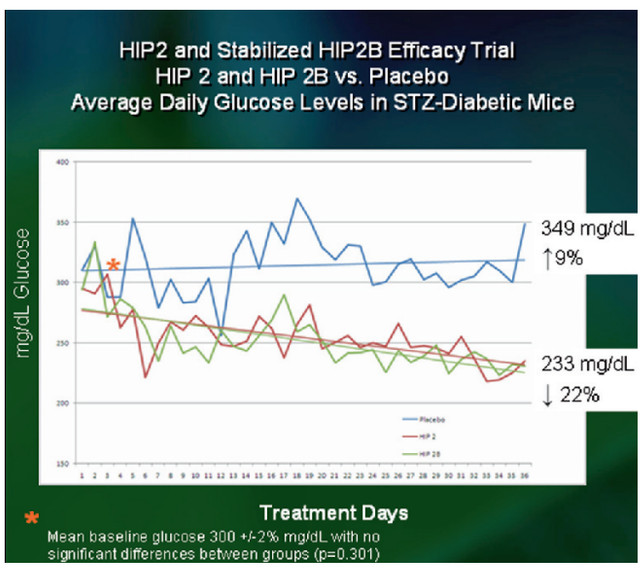
Background and aims: We set forth to compare the impact of a 14 amino acid bioactive region within the human Reg3a gene protein known as Human proIslet Protein (HIP2) on islet neogenesis and glucose-lowering ability to a stabilized form of this protein. The *Reg* gene is upregulated during new onset in type 1 diabetes and among many mammalian species following acute pancreatic injury and may be a potential agent to reverse type 1 and 2 diabetes. Reg3a is hypothesized as an initiating trigger for islet neogenesis and has been shown in animal models to result in formation of new functional islets containing alpha, beta gamma and delta cells. HIP was previously shown to significantly 1) increase insulin levels in human pancreatic ductal tissue and 2) lower glucose levels and increase islet numbers in STZ-rendered diabetic mice.
Materials and methods: The half-life of native HIP2 is 1.2 minutes. We stabilized HIP2 to enhance its potential efficacy as a human drug candidate. HIP was modified by blocking the ends, PEGylation and dimerization with *in vitro* trials conducted in a PANC1 cell line. A candidate was selected based upon improved half-life (20.4 minutes) without evidence of cell damage. *In-vivo* trials were conducted among STZ-rendered diabetic mice. At the end of the 39 day study, mice were fasted for 12 hours.
Results: Placebo-treated mice had significantly higher fasting glucose levels of 258.00 ± 84.5 mg/dL compared to stabilized HIP2B-treated animals with 106.7 ± 0.58 mg/dL (p=0.046). HIP2B treated mice had significantly lower daily random glucose levels compared to controls (Figure 1). The stabilized HIP2B group had a three-fold higher number of islets staining for insulin, glucagon and somatostatin compared to the placebo group (94.00 ± 32.74 and 31.67 ± 15.28 p=0.040). Total islet area was significantly greater in the stabilized HIP2B group compared to placebo (416,714.67 μm^2± 121,389.01 and 127,410.67 μm^2± 96930.78 p=0.032). There were no differences between islet size between the stabilized HIP2B group and placebo (p= 0.518).
Conclusion: HIP2B may address key pathological issues to reverse diabetes. For humans to maintain the success seen in mice may require lifestyle modifications among type 2 patients and concomitant usage of immune therapy among type 1 patients. Human trials with stabilized HIP2B among type 1 and 2 patients will commence in the coming year.



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PS 34 Beta cell signal transduction I

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Growth arrest specific protein 6 (gas6) and its receptors expression and signalling in beta cells
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Background and aims: The growth arrest-specific protein 6 (GAS6) belongs to the family of plasma vitamin K-dependent proteins. It shows homology (~40%) to the plasma anticoagulant protein S, another gamma-carboxylated protein. GAS6 exhibits growth factor-like effects, as it interacts with receptor tyrosine kinase, such as Axl, Tyro3 and Mer. GAS6 and Axl, Tyro3 and Mer have further been implicated in inflammation, cytokine production, immune responses, hemostasis, and cancer. It was therefore of major interest to study GAS6 signalling in islets and clonal beta-cells.
Materials and methods: Glucose-responsive INS-1 832/13 insulinoma beta-cells and human pancreatic islets were used. TaqMan qRT-PCR analysis was applied to measure cytokine mRNA expressions. Immunoblotting and immunocytochemistry were used to detect GAS6 and its receptors in human pancreatic islets and INS-1 832/13 beta-cells as well as GAS6-induced phosphorylation of Axl [pY779] and Akt/PKB [pS473]. To asses further GAS6-stimulated global post-translational protein modifications, especially tyrosin phosphorylation, in INS-1 832/13 beta-cells 2D-PAGE and immunoblotting were combined with stable isotope labeling with aminoacids (13C615N1Leu) in cell culture (SILAC) to quantify the effect of GAS6 by mass spectrometry. To do so, protein samples extracted from 2D-gels were analyzed by MALDI-Trap and LC-MS/MS.
Results: Immunoblotting and immunocytochemistry showed expression of GAS6 and its receptors Axl and Tyro3 but not Mer in human pancreatic islets. Immunoblotting for Axl [pY779] and Akt/PKB [pS473] detected phosphorylation of Axl-receptor and Akt/PKB induced by 400 ng/ml GAS6 in INS-1 832/13 cells. Thus, treatment of INS-1 832/13 beta-cells with GAS6 leads to Axl-receptor mediated PI3-kinase/Akt signalling. Furthermore, the comparison of untreated and GAS6-stimulated INS-1 832/13 beta-cells by 2D-immunoblotting for phospho-tyrosine showed 23 modified proteins. Up to now, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and glucose regulated protein 78 (GRP78/BiP) were identified by mass spectrometric peptide sequencing and changes in GAS6-induced protein expression and tyrosine phosphorylation quantified based on SILAC. These GAS6-induced protein modifications are associated with preventive effect of GAS6 on high glucose-induced increase in TNF-alpha mRNA expression in INS-1 832/13 beta-cells.
Conclusion: We show for the first time that GAS6 and its receptors are present in pancreatic islets and beta-cells and alter TNF-alpha production. The employed proteomic methods revealed that (i) activation Axl/PI3-kinase/Akt signalling and (ii) tyrosine phosphorylation of GAPDH and GRP78, as part of the cellular response to GAS6-stimulation downstream of PI3-kinase and Akt, might be involved in the GAS6 effects on cytokine production. These effects of GAS6 on pancreatic beta-cells demand further investigations.
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Complement receptor C5aR is expressed in pancreatic islets and affects glucose-induced insulin and glucagon secretion
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Background and aims: Complement factors form an important part of the humoral innate immune system and have been implicated in the pathogenesis of autoimmune diseases, including type 1-diabetes. There is however accumulating evidence that the complement system can modulate pancreatic islet function and may also be an important player in the development of type 2-diabetes. For example, patients with type 2-diabetes exhibit increased neutrophil levels of C5a. C5a is produced as a result of complement activation from complement factor C5 and exerts its effect by binding to its receptor C5aR. In this study we have detailed the expression of human complement system in normal islets, and investigated the role of the C5a and C5a receptor antagonist (C5aRant) in human islet function.

Materials and methods: Expression in human islets: Total RNA was isolated from pancreatic islets from 34 non-diabetic donors using the AllPrep RNA Mini Kit and analyzed using Gene 1.0 ST whole transcript based assays. Secretion assay: Insulin and Glucagon secretion was measured by RIA using donor human islets obtained from the Nordic Network of Clincial Islet Transplantation (Prof. Olle Korsgren, Uppsala University) in collaboration with the Lund University Diabetes Center Human Tissue Lab (Dr. Jalal Taneera).
Results: Microarray analysis demonstrated the presence of transcripts of nearly all the complement genes in human pancreatic islets. Next, the effect of C5a on insulin and glucagon secretion was investigated using normal and diabetic human islets. In normal human islets, C5a failed to affect glucose-stimulated insulin secretion (16.7mM). However, addition of C5a together with the C5a receptor antagonist (C5aRant), significantly increased insulin release by (32%; P=0.003; n=38). It was ascertained that the C5aR antagonist alone did not stimulate insulin release. Interestingly, in diabetic human islets C5a and C5aR antagonist did not produce any significant effects on insulin secretion. Similar observations were made for glucagon measurements. In the normal human islets, C5a had no significant effect, whereas in the simultaneous presence of C5a and C5aRant an increase in the amount of glucagon released was observed (37%; P=0.003; n=37). In diabetic islets, the effects on glucagon secretion by C5a and the C5a receptor antagonist were essentially similar to normal islets; C5a alone had no clear effect, whereas addition of the C5a receptor antagonist C5aRant produced a clear increase (57%; P<0.0001; n=12) in glucagon release.
Conclusion: Human islets express all complement factors. The C5a signalling system affects both insulin and glucagon secretion. In addition, C5a signalling in the beta-cell may be altered in type 2-diabetes.
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p140Cas-associated protein (Cap), expressed in beta cell, suppresses insulin secretion
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Background and aims: p140Cap is a multidomain adaptor protein that was first found as an interactive partner of p130Cas (Crk-associated substrates), a protein highly tyrosine phosphorylated in fibroblasts transformed with v-Src or v-Crk oncogene. In non-transformed cells, p130Cas is tyrosine phosphorylated upon ligation of integrins. p140Cap is also tyrosine phosphorylated upon integrin-dependent adhesion or epidermal growth factor signaling, indicating a possible role in cell matrix and in growth factor signaling. In addition, p140Cap was also identified as a synaptosome-associated protein of 25 kDa (SNAP-25)-interacting protein. SNAP-25 plays a major role in membrane docking of synaptic vesicles during neurotransmitter release. Subcellular fractionation analysis suggested that p140Cap interacts with cortical cytoskeleton as well as SNAP-25, it may contribute to neuronal secretion in brain. We had identified a multidomain adaptor protein, vinexine, of which third Src-homology domain interacts with the C-terminal Pro-rich motif of p140Cap with screening study of p140Cap-binding proteins in rat brain. In this study, we examined the possibility whether p140Cap and vinexine might be expressed in the pancreas islet, since proteins involved signal transductions in neurons are often shared with endocrine cells.
Materials and methods: Immunohistological studies using anti-p140Cap antibody and anti-vinexine antibody were performed in rat pancreas. Next, to evaluate the role of p140Cap, the effect of gene silencing using the complex of Lipofectamine 2000 and shRNA on glucose-stimulated insulin secretion was examined in Ins-1 cells and cultured rat insulinoma cells. We studied effect of progression of diabetes mellitus on the expression of p140Cap in Otsuka Long Evans Tokushima Fatty (OLETF) rats, a model of obese type 2 diabetes.
Results: Although p140Cap is associated with vinexine in neurons, p140Cap was detected in beta cells, whereas vinexine in alpha cells in islet. Insulin secretion was elevated in the presence of glucose with time and dose depending manner in Ins-1 cells. Knockdown of p140Cap with shRNA enhanced glucose-stimulated insulin secretion to 260% without increasing in basal insulin level. Silencing of p140Cap resulted in an increase of high potassium-stimulated insulin secretion, but no increase in glucose-stimulated one in MIN6 cells. Our results strongly suggested that p140Cap might negatively regulate insulin secretion in beta cells. Moreover, both immunoreactive insulin and p140Cap were detected in the same cells of pancreas islets in control rat, while poor concordance was observed in OLETF rats. In conclusion, p140Cap and vinexine were recognized in beta cells and alpha cells, respectively.
Conclusion: p140 Cap was expressed in beta cell, which may negatively regulate insulin secretion.

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Expression and function of equilibrative nucleoside transporter 3 in beta cells
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Background and aims: Loss of function recessive mutations in the SLC29A3 gene encoding ENT3 have been identified in a novel diabetes syndrome called "pigmented hypertrichotic dermatosis with insulin-dependent diabetes" (PHID). ENT3 is a member of the equilibrative nucleoside transporter family that function primarily as cellular mediators of nucleoside uptake. It is distinct from other ENTs due to its predominant intracellular localisation. Recently, it was reported that in human hepatocytes and placental tissues, ENT3 is expressed mainly in the mitochondria, to which it transports native nucleosides. This study aimed to investigate the expression and function of ENT3 in pancreatic beta cells.
Materials and methods: ENT3 expression was examined by RT-PCR, Western blotting and immunohistochemistry. The subcellular localisation of ENT3 was determined by exposure of MIN6 beta cells to MitoTracker red CMXRos before subsequent immunostaining with an ENT3 antibody. Cytokine-induced apoptosis was quantified by caspase 3/7 assays in MIN6 cells that had been either exposed to the ENT inhibitor dipyridamole or transfected with ENT3 siRNAs.
Results: PCR amplifications using human and mouse ENT3 primers produced single products of the appropriate sizes from human islet, MIN6 beta cell, mouse islet, and exocrine pancreas cDNAs. Western blotting using an ENT3 antibody detected a 52kDa protein in human islets, mouse islets and exocrine pancreas. A 65kDa protein was detected in MIN6 cells, mouse islets and exocrine pancreas, which most likely represents a post-translationally modified form of ENT3. Immunohistochemistry using archived human pancreas sections demonstrated extensive ENT3 immunostaining of beta cells, which was confirmed by co-staining with an anti-insulin antibody. Furthermore, immunostaining of MIN6 cells that had been exposed to MitoTracker with an ENT3 antibody showed co-localisation of ENT3 to beta cell mitochondria. Inhibition of MIN6 cell ENTs with 10 μM dypridamole resulted in significant increases in basal apoptosis (caspase 3/7 assay luminescence units; dypiridamole-treated vs. control: 47,741 ±2,249 vs. 42,550 ±840; n=8; P <0.05) and in cytokine-induced apoptosis (725,136 ±28,919 vs. 624,147 ±24,279; n=8; P <0.05). More specifically, MIN6 cells transfected with siRNAs directed against mouse ENT3 showed significantly enhanced levels of cytokine-induced beta cell apoptosis compared to control cells transfected with scrambled siRNAs (139,634 ±4,613 vs. 106,426 ±3,001; n=8; P <0.001).
Conclusion: These observations demonstrate that ENT3 is predominantly expressed by islet beta cells, with lower expression levels detected in the exocrine pancreas. Our results also indicate that ENT3 co-localises with mitochondria in MIN6 cells, suggesting a functional role in beta cell physiology. Finally, reduced ENT3 activity or expression is associated with enhanced beta cell apoptosis, which might account for the occurrence of autoantibody-negative insulin-dependent diabetes in individuals with non-functional ENT3 mutations.

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The role of neurexins in the exocytosis of insulin granules from beta cells
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Background and aims: Pancreatic β cells express many protein constituents of the neurotransmitter exocytotic machinery including a family of transmembrane, synaptic adhesion molecules called neurexins (NRXNs). In the brain, α-NRXNs help organize the neuronal exocytotic machinery via extracellular interactions with other synaptic proteins and via intracellular interactions with the exocytotic proteins CASK, Mint, Munc18, and Syntaxin. We hypothesized that α-NRXNs play a comparable role in β cells, participating

in insulin secretory granule docking and exocytosis. The aims of this study were:
1) to characterize NRXN expression in β cells.
2) to determine if NRXN contributes to insulin granule exocytosis.
Materials and methods: Plasma membrane purification, western blot analysis, immunostaining, and absolute RT-qPCR were used to examine NRXN expression and localization in β cells. Immunoprecipitation was used to identify NRXN binding partners in the INS-1E β-cell line. To test whether NRXNs are involved in insulin secretion, we examined the effect on glucose-stimulated insulin secretion of NRXN1 knockdown using siRNA and of NRXN1 overexpression in INS-1E β cells. Knockdown and overexpression experiments were also conducted by measuring glucose-stimulated human growth hormone (hGH) secretion from cells cotransfected with hGH. Constitutive secretion in knockdown experiments was evaluated by cotransfecting NRXN1 siRNA with secreted alkaline phosphatase (SEAP) and measuring SEAP secretion at basal (2.5mM) and high (15mM) glucose. Western blot analysis was used to examine NRXN protein levels in INS-1E β cells after high glucose stimulation.
Results: We determined that NRXN protein is expressed on the β-cell surface and is not present in other islet cell types. NRXN1α and 2β are the most abundant NRXN isoforms in the β cell, with transcript levels comparable to those in brain. CASK, Syntaxin1 and Munc18 coimmunoprecipitated with NRXN1 from INS-1E β cells. Decreased NRXN1 expression after siRNA treatment of INS-1E cells resulted in a 54% increase ($p<0.05$) in insulin secretion at high glucose but had no effect on basal insulin secretion. This result was confirmed by measuring secretion of transfected hGH, which is packaged and secreted in insulin-containing secretory granules. NRXN1 knockdown did not affect constitutive secretion. Increased levels of NRXN1 had no effect on secretion at high glucose but resulted in a slight (24%) but consistent increase in basal secretion. After 1h of glucose stimulation, NRXN1 protein levels decreased by 33% in non-transfected and 40% in NRXN1-overexpressing INS-1E β cells ($p<0.05$).
Conclusion: We conclude that NRXN1 is an integral component of the β-cell submembrane secretory machinery. NRXNs are involved in the exocytosis of insulin granules from β cells, possibly by organizing the exocytotic machinery and/or by contributing to the granuphilin-mediated docking of granules at the β-cell surface. Increased insulin secretion after NRXN1 gene silencing and the decrease in NRXN1 levels observed during glucose stimulation suggest that NRXN1, like granuphilin, contributes to the negative regulation of insulin release. Future work will determine the role of NRXNs in the docking, priming and fusion steps of insulin granule exocytosis.
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Neuroligin-2 increases insulin expression and secretion in pancreatic beta cells via extracellular binding interactions
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Background and aims: The pancreatic β-cell secretory apparatus is similar to that used by neurons for synaptic exocytosis. Many of the scaffolding and vesicular proteins important for neurotransmitter secretion are key components of the β-cell insulin secretory machinery. In neurons, extracellular interactions of neuroligin proteins with either neurexins or other synaptic proteins help to drive the recruitment of the exocytotic machinery to the axonal membrane. These transmembrane, synaptogenic adhesion molecules are of particular interest because a specific subset is expressed by β cells and because neuroligin-2 (NL-2) has been implicated by our group in the regulation of insulin secretion. These results along with previous studies demonstrating a role for neuroligins in the maintenance and differentiation of the neurotransmitter release machinery in the brain and the importance of cell-cell contacts in the regulation of insulin secretion led us to test the hypothesis that NL-2 helps to drive the assembly of the β cell secretory apparatus through extracellular interactions that facilitate β cell maturation and regulated insulin secretion.
Materials and methods: Antibodies targeted to the NL-2 extracellular domain and recombinant soluble NL-2 were utilized to test for the presence of NL-2 protein and NL-2 binding partners on the β cell surface. The role of extracellular interactions involving NL-2 in contact-enhanced stimulus-secretion coupling and β-cell insulin expression was tested by assessing insulin secretion and content after co-culturing MIN-6 and INS-1E β cells with other cells (HEK293) transfected with NL-2 or control expression constructs. Soluble NL-2 protein was utilized to disrupt extracellular protein interactions in rat islets and MIN6 cell cultures. The binding of radioiodinated, recombinant NL-2 to the surface of intact β cells was analyzed by determination of competitive binding curves.
Results: Fluorescence flow cytometry reveals that NL-2 is expressed on the surface of INS-1E and MIN-6 β cells. Binding of NL-2 to the β cell surface was also detected, with INS-1E and MIN6 β cells each yielding two distinct populations with different binding properties. The dissociation constant (K_d) of NL-2 with its binding partner on the β-cell surface is 15 nM, lower than would be expected if the binding partner were neurexin. Addition of soluble NL-2 to the culture media of isolated rat islet or INS-1E β cells reduces insulin secretion by up to 70% in a concentration-dependent manner. Half-maximal inhibition of insulin secretion is achieved with 9 nM soluble NL-2, a concentration approximately the same as the K_d of the binding interaction. Coculture of β cells with cells expressing neuroligin-2 increased stimulated insulin secretion by 37% ($p<0.01$) and cellular insulin content by up to 40% ($p<0.05$).
Conclusion: NL-2 is expressed on the β cell surface and binds with high affinity to another β-cell surface protein. Extracellular interactions involving NL-2 increase insulin expression and secretion by β cells. These results are consistent with the hypothesis that NL-2 promotes the development and maintenance of the insulin secretory machinery in β cells through trans-cellular interactions.
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Inhibition of beta-secretase activity affects pancreatic beta cell function
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Background and aims: BACE1 and BACE2 (ß-site APP-cleaving enzyme 1 and 2) are proteases sharing typical structural features with type I membrane associated aspartyl proteases. Both enzymes have been found to be differently expressed in various human tissues and, in particular, BACE1 appears to be more abundant in the brain, where it is involved in the pathogenesis of Alzheimer's disease as the ß-secretase generating the ß-amyloid peptide. In all amyloidogenic diseases, including Alzheimer's Disease and Diabetes, abnormally folded and insoluble proteins accumulate within or around cells and interfere with their function. Although the ß-secretases BACE1 and BACE2 have also been found in the pancreas, their functional role in this tissue still remains unknown. This report is aims to characterize the localization of BACE proteins in the human pancreas and to determine their role on pancreatic function.
Materials and methods: The enzymatic activity of BACE was detected in protein extracts of the human pancreas with a fluorometric ß-secretase proteolytic activity assay. Immunoblot and immunohistochemistry with specific antibodies in paraffined sections were used to determine the human pancreatic cell types expressing BACEs. The intracellular localization of BACE1 and BACE2 was assessed by the immunocolocalization with specific intracellular markers (Na^+/K^+-ATPase, clathrin, insulin, Gm130, Tfrc) in MIN-6 cell line. To study the role of BACE on MIN-6 cells, we used a selective and fast cell-permeable substrate-based inhibitor designed from the ß-secretase cleavage sites (BI-II). Real time-PCR was used for *insulin* transcript quantification.
Results: High levels of BACE enzymatic activity were detected in protein extracts of human pancreatic islets and exocrine tissue. In human, BACE1 is expressed by both *a* and ß pancreatic cells population as well as in the exocrine tissue, while BACE2 is restricted to islet ß-cells. The intracellular localization of BACEs in MIN-6 cells showed BACE1 colocalization with insulin and BACE2 expression in clatrin-coated endocytic vesicles of the plasma membrane. When BACE pharmacological inhibition (BI-II) was performed for 24h in MIN-6 cells, BACE2 content in plasma membrane and clathrin-coated vesicles was significantly increased compared to control (from 4.8±0.8 to 9.7±2.6%, $p<0.05$ and from 14.0±2.6 to 26.2±1.9%, $p<0.001$ respectively), suggesting a BACE2 involvement in the processes of receptor-mediated endocytosis and recycling by clathrin-coated vesicles. The analysis of insulin internalization rate after glucose stimuli is reduced in BI-II treated MIN-6 cells compared to control (0.23±0.02 to 0.30±0.02%, $p<0.05$) despite of significantly increased levels of IRß protein extracts. The immunofluorescence

analysis showed a significant decrease in receptor expression at the plasma membrane (Na^+/K^+-ATPase) of treated cells compared to control (from 4.9±1.9 to 0.3±0.01%, $p<0.05$), with the receptor pool being retained in the Golgi apparatus (Gm130). Coherently with the impaired IRß trafficking to the plasma membrane, we also observed a 1,4 fold reduction in the transcriptional activity of the *insulin* promoter.
Conclusion: Our data suggest that the ß-secretes activity is needed for *insulin* expression in ß-cells and that the BACE2 is the enzyme involved. Thus, BACE2 is here presented as a potentially essential enzyme for ß-cell function.
Supported by: FIS

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Transgenic mice expressing an intestine-specific secretory protein, IBCAP, demonstrates pancreatic beta cell augmenting activity
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Background and aims: Recent success with GLP-1 analogs and DPP IV inhibitory drugs in the clinical application for diabetic patients has highlighted the role of intestine as a hormone producing organ. Crucial roles of these hormones, including GLP-1, GIP and Ghrelin, in the control of energy metabolism and food intake, and their relation with the metabolic syndrome have been brought to worldwide attention. In the current studies, we aimed to search for secretory proteins expressed in the intestine.
Materials and methods: We have constructed and screened a mouse intestinal cDNA library to search for genes encoding secretory and membrane proteins, using the Oligo-cap Signal Sequence Trap (Oligo-cap SST) method developed in our laboratory. For CF266 functional analysis *in vivo*, we prepared the recombinant CF266 expressing adenovirus and we analyzed the phenotype of CF266 over-expression mice model.
Results: We have identified CF266 as a novel intestine-specific secretory protein using the Oligo-cap SST strategy. We demonstrated that CF266 had insulin secretion promoting effect, and furthermore, that adenovirus-mediated expression of CF266 in STZ-treated type 1 diabetes model mice improved the blood glucose level of the animal, and showed the increased pancreatic β-cells detected by the histological analysis. Here, we have developed transgenic (Tg) mice expressing CF266 under the control of CAG-promoter. Analyses of the Tg mice have shown marked increase of pancreatic islets, confirming our former findings with the STZ-induced diabetic mice treated with CF266 expressing adenovirus. Thus, we renamed CF266 as IBCAP; intestine-derived beta-cell augmenting promoter. Further analyses have shown that blood glucose concentration and OGTT of IBCAP Tg mice are relatively normal compared with the control mice. Therefore, IBCAP seems to have promoted the augmentation of islets which are functionally normal. We are now testing whether augmentation of the β-cell islets is due to inhibition of apoptosis or stimulation of proliferation.
Conclusion: Our findings will provide IBCAP as another potential therapeutic target for diabetes and pancreatic β-cell regeneration.
Supported by: Ministry of Education, Culture, Sports, Science and Technology of Japan

PS 35 Beta cell signal transduction II

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Increased phosphorylation of FOXO1 during glucocorticoid excess is not mediated by SGK1 (Serum glucocorticoid inducible kinase 1) in insulin secreting cells
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Background and aims: The essential role of insulin receptor signalling including PI3K and PKB activation for sufficient insulin disposal is well documented. Previously, we demonstrated that glucocorticoids induce the expression of serum- and glucocorticoid-inducible kinase 1 (SGK1), an enzyme with 54 % identity in the catalytic domain with PKB and stimulated by insulin. SGK1 in parallel to PKB exerts anti-apoptotic effects in a variety of cells. Indeed, unlike glucocorticoids, transfection with SGK1 did not augment apoptotic cell death of insulin secreting INS-1E cells. The aim of the present study was to examine whether FOXO1, a PKB substrate, is regulated by SGK1 in cells under glucocorticoid excess.
Materials and methods: Insulin secreting INS-1E cells were treated with dexamethasone (dexa, 100 nM) to induce the endogenous expression of SGK1. SGK1 activity was selectively inhibited with a specific inhibitor (GSK650394) or by transient transfection with siRNA against SGK1. Alternatively, cells were transiently transfected with constitutive active or dominant negative hSGK1. Expression and phosphorylation of proteins were analyzed by Western blotting. Cellular distribution of immunostained proteins was examined using confocal microscopy.
Results: Treatment of the cells with dexa reduced phosphorylation of PKB, but paradoxically increased phoshorylation of FOXO1. The inhibition of PKB by Akti-1/2 abolished phosphorylation of FOXO1 in control cells. In dexa-treated cells FOXO1 phosphorylation was inhibited by Akti-1/2 but not by SGK1 inhibitor (up to 10 μM). In parallel, Akti-1/2 promoted nuclear translocation of FOXO1 while SGK1 inhibitor did not. Pretreatment of cells with siRNA against SGK1 inhibited the induction of SGK1 by dexa by 66 % at the mRNA level and by 80 % at the protein level. Although SGK1 protein level was reduced dexa treatment still increased FOXO1 phosphorylation. Furthermore, in cells transfected with either constitutive active or dominant negative hSGK1 phosphorylation of FOXO1 was unchanged.
Conclusion: These data suggest that the increased phosphorylation of FOXO1 observed after dexa-treatment is sensitive to PKB inhibition but does not depend on SGK1 in INS-1E cells.
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PLCζ expressing in islet beta cells: does it express in sperm specifically?
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Background and aims: Phospholipase C (PLC) is considered to modulate insulin secretion stimulated by diverse factors such as nutrients, hormone, neurotransmitter and ions. There are six established families of PLC termed β, γ, δ, ε, ζ andη, however, the role of each isoform in stimulated insulin secretion remains obscure. In this study, several isoforms of PLC which could express in pancreatic beta cell were identified. And the expression level of each isoform after stimulated by different factors was quantified in order to reveal the function of each isoform in insulin secretion.
Materials and methods: The expression levels of the isoforms of PLC in INS-1 cells (a rat insulinoma cell line) were semi-quantified by RT-PCR. In order to study on the role of each isoform in different factor-stimulated insulin secretion, glucose, L-aminoglutaminic acid (L-GLU), chloratum kalium (KCL), cholecystokinin-octopeptide (CCK8), and acetylcholine chloride were chosen as stimulating factors. INS-1cells were stimulated by the factors mentioned above respectively, and the mRNA level of each PLC isoform was semi-quantified by RT-PCR.
Results: Surprisingly, PLCζ, an isoform of PLC which was thought to express specifically in sperms was detected in INS-1 cell line and rat pancreatic tissue in our study. The expression of PLCδ, PLCβ, PLCγ and PLCη could also be detected in INS-1 cells except for PLCε. Many isoforms were induced when INS-1 cell line stimulated by different factors. PLCδ (1.97 folds), PLCβ and

PLCγ expressions on INS-1 cells were significantly up-regulated after stimulation with glucose (P<0.05); PLCβ (1.98 folds), PLCδ and PLCζ expressions on INS-1 cells were significantly up-regulated after stimulation with L-GLU (P<0.05); PLCη(1.89 folds), PLCδ, PLCγ and PLCζ expressions on INS-1 cells were significantly up-regulated after stimulation with KCL (P<0.05); PLCδ (2.54 folds), PLCγ and PLCη expressions on INS-1 cells were significantly up regulated (P<0.05) after stimulation with CCK8; And the expression of PLCδ was increased 1.84 folds when INS-1 cells were stimulated by acetylcholine chloride(P<0.05).
Conclusion: This is the first study to demonstrate that PLCζ expresses in islet beta cells. The expression of PLCδ was significantly increased while INS-1 cells were stimulated by glucose, CCK8 and acetylcholine chloride; The expression of PLCβ was significantly increased while INS-1 cells were stimulated by L-GLU; and the expression of PLCη was significantly increased while INS-1 cells were stimulated by KCL. Different isoforms of PLC were differentially expressed while INS-1 cells under different pressures, indicating that different stimulation factor stimulate INS-1 cell to secrete insulin through different isoforms of PLC.
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PKCdelta modulates cell cycle progression and survival through the regulation of cytosolic-nuclear trafficking of p21 in insulin secreting INS-1E cells
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Background and aims: The main cause for overt type 2 diabetes mellitus is decreased beta-cell mass due to increased apoptosis and reduced proliferation. Accumulating evidences suggest the involvement of PKCdelta in apoptotic signalling of beta-cells but little is known about the role of this kinase for proliferation. Regulation of cell cycle through PKCdelta-dependent modulation of p21 protein was described in several cell systems. $p21^{WAF1/CIP1}$ is an inhibitor of several cyclin-dependent kinases in the nucleus. It has been described that PKCdelta- and PKB-dependent phosphorylation of p21 affects its cytosolic-nuclear trafficking. In pancreatic beta-cells the role of p21 is still under debate. While the p21 KO mice have normal beta-cell mass and replication rate, the over-expression of p21 in beta-cells led to increased apoptosis but surprisingly conferred improved recovery from streptozotocin-induced diabetes. This study was undertaken to elucidate the role of PKCdelta and p21 for cell cycle progression and survival of beta-cell.
Materials and methods: INS-1E cells were stably infected with wild type (PKCdeltaWT) or kinase dead (PKCdeltaKN) PKCdelta using a retroviral system. Cell cycle was analysed after staining nuclear DNA with propidium iodide using fluorescence activated cell sorting (FACS). Proteins expression was assessed by Western blotting and their subcellular distribution by Western blotting after nuclear and cytosolic fractionation of cells or by confocal microscopy after immunostaining for the respective proteins. Apoptosis was quantified by TUNEL staining.
Results: PKCdeltaKN cells displayed reduced growth and increased apoptosis (7.8 %), while growth and apoptosis (4.5 %) of PKCdeltaWT cells were similar to those of control INS-1E cells (3.7 %). The analysis of cell cycle phases revealed that PKCdeltaKN cells have a strongly impaired G2/M transition accumulating in G2, whereas the cell cycle distribution pattern of PKCdeltaWT cells did not differ from control INS-1E cells. Further, 17.4 % of the PKCdeltaKN cells stained positive for the G2/M marker p-ser10-histone H3 but only 5.8 % of PKCdeltaWT and 8.3 % of control INS-1E cells were histone H3-positive. The G2 cell cycle regulatory protein cdc2 and the inhibitor p27 were expressed at similar levels in control, PKCdeltaWT and PKCdeltaKN cells, whereas p21 protein was reduced in PKCdeltaWT cells. The analysis of subcellular distribution showed extrusion of p21 from the nucleus in PKCdeltaWT cells. In control cells 8.1 % of nuclei and in PKCdeltaKN cells 24.2 % of nuclei stained positive for p21. Inhibition of the nuclear export machinery with leptomycin B neither induced nuclear accumulation of p21 in PKCdeltaWT cells nor augmented the accumulation of p21 in PKCdeltaKN nuclei.
Conclusion: These observations suggest that PKCdelta inhibits nuclear entrance of p21 and the cytosolic trapping of p21 sustains cell cycle progression. On the other hand, in PKCdeltaKN cells increased nuclear accumulation of p21 may lead to prolonged G2 phase and impaired cell cycle progression affecting cell growth and survival.
Supported by: DFG

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Knock-down of PI3K-C2α leads to increased proliferation of pancreatic beta cells
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Background and aims: PI3K-C2α plays an important role in controlling cell survival via the intrinsic cell death pathway. Knock-down of PI3K-C2α protein levels led to a reduction in cell proliferation and viability in a number of cancer-derived cell lines. We have recently shown that PI3K-C2α generates $PI(3,4)P_2$ in beta cells and is involved in glucose-stimulated insulin release. Knock-down of PI3K-C2α levels leads to reduced PKBα activity, AS160 phosphorylation and glucokinase protein levels. The aim of this study was to evaluate the role of PI3K-C2α in beta cell proliferation and survival.
Materials and methods: MIN6 cells and primary mouse beta cells were treated with control siRNA or siRNA against PI3K-C2α. Knock-down of protein levels of PI3K-C2α was verified by Western blotting (80% knock-down). Proliferation in MIN6 cells was measured directly by counting the number of cells and by BrdU-incorporation. Apoptosis rate of MIN6 cells after treatment with either H_2O_2 or staurosporine was determined by triple staining with Hoechst 33342, propidium iodide (PI) and AlexaFluor488-annexinV, where AlexaFluor488-annexinV positive PI-negative stained cells were considered apoptotic. Cell proliferation of primary mouse beta cells was determined by BrdU-incorporation. Phosphorylation of PKB target proteins and Raf-1 in MIN6 cells was evaluated by Western blotting.
Results: To our surprise knock-down of PI3K-C2α did not lead to an inhibition of cell growth like in other cell lines, but to an increased cell proliferation. Cell count 120 h after siRNA transfection gave a 3.2 ± 0.12fold increase in cell number in PI3K-C2α siRNA-treated cells compared to control siRNA-treated MIN6 cells. BrdU incorporation was increased to 21.75 ± 2.54% in PI3K-C2α siRNA-treated MIN6 cells (9.38 ± 1.06% in control siRNA-treated cells) and to 1.67 ± 0.22% in primary mouse beta cells treated with PI3K-C2α siRNA (0.71 ± 0.067% in control siRNA-treated cells). Knock-down of PI3K-C2α led to a significant (p<0.05) protection against apoptosis induced by 20μM H_2O_2 (11.95 ± 1.86 % apoptotic cells in PI3K-C2α siRNA-treated MIN6 cells versus 24.19 ± 0.89 % in control siRNA-treated cells) or by 6μM staurosporine (24.89 ± 1.37 % apoptotic cells in PI3K-C2α siRNA-treated MIN6 cells versus 34.61% ± 2.42 % in control siRNA-treated cells). Since insulin-stimulated PKBα activity was diminished in PI3K-C2α siRNA-treated cells, we evaluated the phosphorylation of PKB target proteins (TSC2, GSK3β, FoxO1) in MIN6 cells after insulin stimulation. GSK3β Ser9-phosphorylation was not altered by PI3K-C2α-siRNA treatment. The increase of TSC2-Thr1462- and FoxO1-Ser256-phosphorylation after insulin stimulation was abolished by PI3K-C2α siRNA-treatment. Ser338-phosphorylation of Raf-1 was increased in PI3K-C2α siRNA-treated MIN6 cells after insulin stimulation (1.54 ± 0.12fold compared to control siRNA-treated cells). The observed changes in the phosphorylation status of TSC2 and FoxO1 are in line with diminished PKBα activation in response to insulin stimulation, but would lead to inhibition of cell growth, rather than to its stimulation. The increased phosphorylation of Raf-1, a kinase of the Ras/Raf-1/ERK cascade known to be involved in beta cell proliferation and protection against apoptosis, could explain the increased proliferation in PI3K-C2α siRNA-treated cells.
Conclusion: Knock-down of PI3K-C2α in pancreatic beta cells leads to increased cell proliferation by a pathway involving Raf-1 activation.
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Focal adhesion remodelling is crucial for glucose-stimulated insulin secretion and involves activation of focal adhesion kinase and paxillin
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Background and aims: Despite the numerous studies on the mechanism of pancreatic beta cell insulin secretion, many aspects of the molecular machinery remain to be elucidated. Actin cytoskeleton remodelling is well known to be positively involved in glucose-stimulated insulin secretion (GSIS). We have observed glucose-stimulated changes at the beta cell surface that are similar to focal adhesion remodelling in cell migration. This led us to study the role of two key focal adhesion proteins, Focal Adhesion Kinase (FAK) and paxillin, in glucose-stimulated beta cell focal adhesion remodelling and insulin secretion.

Materials and methods: FACS-sorted rat primary beta cells were used unless stated otherwise. For immunofluorescence staining and protein phosphorylation, beta cells were incubated for 2h at 2.8 mM glucose followed by 16.7 mM glucose for up to 1h. siRNA was used to knockdown paxillin expression: cells were transfected (Lipofectamine 2000; 100nM siRNA) 3 days prior to testing GSIS [2h pre-incubation at 2.8 mM glucose; 1h 2.8 mM glucose (basal); 1h 16.7mM glucose (stimulated)]. Data are mean±SEM, n=3 independent experiments.
Results: In the basal state, FAK (Tyr-397) and paxillin (Tyr-118) were modestly phosphorylated and localised in few long filopodia at the basal cell surface. Stimulation for 20 min at high glucose resulted in increased phosphorylation of FAK and paxillin and cell spreading, with a 59±8% ($p<0.001$) increase in cell surface area vs. basal after 1h at high glucose. Within 20 min at 16.7 mM glucose there was appearance at the basal surface of numerous newly formed, shorter actin filopodial extensions, containing phosphorylated paxillin. Co-incubation with the L-type Ca^{2+} channel blocker, SR-7037 (10μM), completely inhibited this sequence of events, indicating requirement of increased cytosolic Ca^{2+}. Furthermore, knockdown of paxillin (45±2% decrease by western blot) decreased GSIS by an average of 61±4% ($p<0.05$) vs. control transfected with scramble RNAi. A 43±1% ($p<0.05$) decrease in GSIS was observed following pharmacological inhibition of glucose-induced phosphorylation of FAK Tyr-397 by compound Y15 in the MIN6B1 beta cell line; this was confirmed in a single preliminary experiment on primary rat beta cells.
Conclusions: We show here that glucose-stimulated spreading of beta cells coincides with the remodelling of filopodial extensions and phosphorylation of the two main focal adhesion proteins, FAK and paxillin. Additionally, GSIS was significantly decreased by paxillin knockdown and inhibition of FAK activation, showing for the first time that focal adhesion remodelling is a critical event in pancreatic beta cell function.
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Novel aspects of female sex hormone estrogen in diabetes
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Background and aims: The lower prevalence of diabetes in females suggests that female sex steroids protect from β-cell injury. Consistent with this hypothesis, 17β-estradiol manifests antidiabetic actions in humans and rodents. Our recent findings show that the stimulatory action of 17β-estradiol on insulin secretion is mediated by the G protein coupled receptor 30 (GPR30), raising the prospect that antidiabetic action of 17β-estradiol might be due, in part, by an antiapoptotic effect on β-cells exerted through activation of GPR30.Therefore,the objective of this study was to identify expression of GPR30 in human pancreatic islets and to further clarify the role of GPR30 in pancreatic hormone secretion and beta cell survival.
Materials and methods: GPR30 expression was analyzed by confocal microscopy, Western blot and qRT-PCR in human pancreatic islets from female and male donors. Hormone secretion and cAMP content in islets were determined with RIA and apoptosis with the Annexin-V method.
Results: Confocal microscopy revealed GPR30 expression in alpha, beta and delta cells of pancreas. GPR30 mRNA and protein expression was markedly higher in female vs male islets ($p<0.01$). Dose-response studies of G-1 (a selective agonist of GPR30) vs 17β-estradiol in isolated islets at 12 mM glucose showed an almost similar pattern in potentiating insulin and suppressing glucagon and somatostatin secretion. The 17β-estradiol genomic receptor (ERα and ERβ) antagonist ICI-182,780 (Fulvestrant) or Acolbifene (EM-652) did neither influence the amplifying effects of G-1 or 17β-estradiol on cAMP content ($p<0.001$) nor insulin secretion from islets. Cytokine-induced (IL1β+TNFα+INFγ) apoptosis in islets, cultured for 24 h at 5 mmol/l glucose, was almost abolished by G-1 or 17β-estradiol treatment ($p<0.001$).These beneficial effects of G-1 or 17β-estradiol on pancreatic islets were not affected by either Fulvestrant or Acolbifene.
Conclusion: In view of these novel finding we suggest that drugs that could selectively modulate the activity of GPR30, represent a promising frontier in diabetes mellitus co-adjuvant therapy.
Supported by: Swedish Research Council

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Polyamines and human pancreatic beta cells
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Background and aims: Polyamines (PA, in particular putrescine, spermidine and spermine) are ubiquitous chemical entities that play an important role in cell function and turnover, as well as in the synthesis of proteins and nucleic acids. Aim of the study was to assess whether PA may be of importance in affecting beta-cells in non-diabetic subjects and type 2 diabetic individuals.
Materials and methods: Pancreatic tissue obtained from 17 type 2 diabetic subjects (T2DM; age: 67±9 years; M/F:11/6; BMI: 29±5 kg/m^2) and 15 non-diabetic controls (ND; age: 62±15 years; M/F:11/4; BMI: 26±2 kg/m^2) was studied. The presence of PA in beta-cells was assessed on electron microscopy by immunogold technique. Gene expression studies were performed with isolated islets or beta-cell enriched samples prepared by laser capture microdissection. Islets insulin secretion was determined in response to acute glucose stimulation after 24 h incubation with or without 2-difluoromethylornithine (DFMO, an inhibitor of ornithine decarboxylase I, 5 mM), putrescine (50 μM) and spermidine (50 μM), alone or in combination.
Results: Putrescine and spermine were detected in beta-cells from ND and T2DM by electron microscopy. Localization was mainly in the cytoplasm for putrescine and in both the cytoplasm and the nucleus for spermine. Microarray analyses showed that ornithine decarboxylase I (key enzyme in PA synthesis) was lower in beta-cells ($p<0.001$) and islets ($p=0.03$) from T2DM. Data were confirmed by qPCR studies, which detected also a lower expression of arginase II in diabetic samples. DFMO caused a decrease ($p<0.05$) of glucose-stimulated insulin release (-32 ± 9.5%); the same was observed in presence of spermidine (-48 ± 32%, $p=0.05$). Putrescine prevented the inhibitory effect of spermidine.
Conclusion: This study shows that 1) PA are present in human beta-cells; 2) PA biosynthesis pathway may be different in type 2 diabetic beta-cells; and 3) PA may have a role in insulin secretion.
Supported by: Italian Society of Diabetes

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Insulin secretion is affected by disruption of mini-P-glycoprotein
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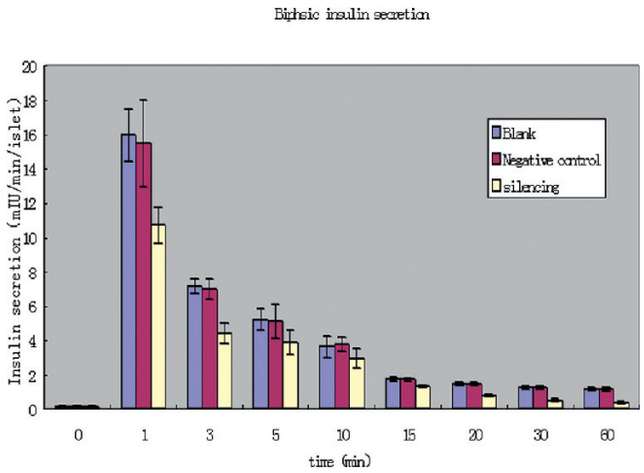
Background and aims: A 65-kDa mdr1-like protein, which exhibited biological functions similar to the sulfonylurea receptor, had been preliminary identified as a mini-P-glycoprotein in rat islets. In this study, the mini-P-glycoprotein was down-regulated by siRNA technique and effects on biphasic insulin secretion were determined.
Materials and methods: Stealth ™ / siRNA duplex oligoribonucleotides (Invitrogen) was designed to silence the *abcb1b* gene (*mdr1,* encoding P-glycoprotein). Stealth RNAi Negative Control Duplexes was severed as negative control. The silencing effects were determined by fluorescence microscopy, quantitative PCR and Western blot. Biphasic insulin secretion was investigated in rat islet batch incubations in three different groups: silencing, negative control and blank (without oligoribonuccleotides). In parallel, apoptosis in the rat islets in the respective groups was also evaluated.
Results: After 48 hours incubation, more than 95% of the islets were visibly transected. The expression of the *abcb1b* mRNA was decreased significantly by the siRNA but not the *abcb1a* mRNA, which were used as technique control ($P<0.001$ and $P=0.815$, respectively). Mini-P-glycoprotein expression was detected using the specific antibody C219 and was reduced by more than 80%. Biphasic insulin release was dramatically reduced by the silencer, both in the first and second phase, when compared with the negative control or the blank groups ($P<0.05$ and 0.001; n=10 for all the groups, Fig.1). Apoptosis was induced when islets were cultured in high glucose (16.7mmol/L) culture medium for 48 hours compared to those in normal culture medium. Up-regulated expression of *casp3* and *bax* were detected in the silencing group ($P<0.01$ and 0.001 *vs* the negative control group or blank group), while *Bcl-2* expression was down-regulated ($P<0.001$). However, the expressions of *casp3*, *bax* and *bcl-2* were not influenced by the silencing of the mini-P-glycoprotein.
Conclusion: The mini-P-glycoprotein may contribute to biphasic insulin secretion. Apoptosis of rat islets is not induced by the disruption of the mini-

P-glycoprotein. It has been suggested that the mini-P-glycoprotein might function as one of the regulatory proteins to the chloride transport protein 3 to mediate insulin granules acidification and priming. Further investigation need to be done to evaluate the underlying mechanisms.



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PS 36 Receptors, secretagogues and modelling in islets

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CART is overexpressed in beta cells of diabetic mice to improve insulin secretion

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Background and aims: Cocaine- and amphetamine-regulated transcript (CART) is a novel islet regulatory peptide. CART null mutant mice exhibit impaired glucose tolerance due to islet dysfunction, and diminished expression of PDX-1. CART regulates islet hormone secretion, including augmenting GLP-1-enhanced glucose-stimulated insulin secretion (GSIS) in vitro. Moreover, CART is upregulated in the beta cells of Type-2 diabetic (T2D) rats. Although the effects of exogenously added CART on the beta cell are established, the role of endogenous beta cell CART is unknown. Furthermore, CART expression in islets of T2D mouse models has not been studied. We studied islet expression of CART in diabetic db/db and ob/ob mice using immunocytochemistry and in situ hybridization. To mimic the situation in beta cells of T2D models we overexpressed CART in clonal INS-1 (832/13) beta cells using adenovirus and measured: 1) Insulin secretion stimulated with glucose (GSIS) and an array of secretagogues. 2) Gene expression of key genes related to insulin secretion and beta cell function. 3) Regulation of CART gene and protein levels after culture in different glucose concentrations.
Materials and methods: Male db/db, ob/ob, and C57BL/6 mice were used. CART expression was examined with immunocytochemistry, in situ hybridization, immunogold labeling and transmission electron microscopy (TEM), real-time PCR and Western blot. preproCART was overexpressed in INS-1 (832/13) cells and insulin secretion after 1h static incubations was analyzed using RIA. GFP-adenovirus was used as negative control.
Results: CART was robustly upregulated in beta cells of both db/db and ob/ob mice, compared to control mice. Immunogold labeling for CART revealed that CART was located to the beta cell granules. CART overexpression in INS-1 (832/13) cells was verified using real-time PCR and western blot. Overexpression of CART resulted in a moderate and dose-dependent augmentation of GSIS. Furthermore, overexpression of CART resulted in increased forskolin-enhanced GSIS. On the other hand, overexpression of CART was without effect on GSIS in the presence of 35mM KCl. Moreover, overexpression of CART elevated mRNA expression levels of insulin, PDX-1 and syntaxin 1A. On the other hand, CART had no effect on the mRNA expression levels of GLUT2, Kir6.2, or Munc-18-1. Culturing CART-overexpressing cells for 24 h in 16.7 mM glucose provoked a decrease in CART mRNA expression and an upregulation of CART protein levels, compared to culture in 3 mM and 11.1 mM glucose.
Conclusion: We conclude that CART is upregulated in the beta cells of diabetic mice and that beta cell CART is regulated by glucose. Furthermore CART stimulates the triggering pathway of insulin secretion, as well as expression of insulin and genes important for beta cell function and insulin exocytosis. Together these data suggest that CART is upregulated in beta cells of T2D animals to improve insulin secretion and gene expression. The potential for CART-based substances for future treatment of T2D remains to be established.
Supported by: The Novo Nordisk Foundation, Swedish Medical Research Council

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Stimulation of 5-HT1a receptor decreased insulin secretion in islets of Langerhans from mice and humans

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Background and aims: 5-Hydroxytryptamine, also known as serotonin activates the receptors 5-HT1a (5-hydroxy-tryptophane receptor 1a) and 5-HT1a (5-hydroxy-tryptophane receptor 2b). 5-HT1a and 5-HT2b are both G-protein coupled receptors, 5-HT1a couples negatively to adenylate cyclase while 5-HT2b activates Gq and activates the phospholipase C pathway. The

G-protein activation result in different cellular events by two distinct intracellular pathways, affecting the levels of calcium in the cell. Both receptors have previously been found in islets of Langerhans from rodents. In addition, serotonin has been shown to be present in islet cells from several different animal species. As both receptors and amines are present, serotonin may potentially regulate hormone secretion from islets of Langerhans. In the previous studies we have shown that a selective 5-HT2b agonist alpha-Methyl serotonin potentiates glucose stimulated insulin secretion from INS-1 cells, mouse, and human islets of Langerhans from healthy individuals. This has now been further investigated in human islets of Langerhans from type 2 diabetic individuals. We have also investigated the effect of a 5-HT1a receptor agonist on insulin secretion in islets of Langerhans from both mouse and humans.
Material and methods: We used RT PCR and sequence specific primers to detected expression of the receptors in the beta-cell line (INS 832/13) and in islets of Langerhans. Immuno-histochemical analysis was used to detect the receptors at the protein level in rodent islet and human islets. Islets from mouse were isolated by standard collagenase digestion. Human and mice islets were incubated with glucose and Buspirone or alpha-methyl serotonin at 37 ºC for 1h and assayed for insulin secretion. Insulin was measured with insulin ELISA specialized for mice and human islets.
Results: 5-HT2b mRNA was found in the 832/13 cells. We also found both 5-HT1a and 5-HT2b to be expressed in rodent and human islets, at both mRNA and protein level. Interestingly, 5-HT receptors in islets were localized in two different cell types in the islets. In rodent islets, 5-HT2b was predominantly expressed in beta-cells while 5-HT1a was more abundant in the alpha-cells. In human, islets the situation was reversed. While stimulation with alpha-methyl serotonin potentiated glucose stimulated insulin secretion in human islets of Langerhans from type 2 diabetic individuals. Buspirone showed a significant decrease of insulin secretion at stimulatory glucose concentrations in mouse and human islets.
Conclusion: Our results strongly suggest that serotonin and the 5-HT1a and 2b receptors regulate insulin secretion. As both 5-HT1a and 5-HT2b are expressed in rodent and human islets and activate two very distinct cellular pathways, these receptors may in fact modulate secretion from both alpha and beta-cells. In conclusion, pathways of serotonin and its receptors may provide exciting new insight in the regulation of islet hormone secretion.
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Nesfatin-1 stimulates insulin secretion, inhibits glucagon secretion and is expressed in human and rodent beta cells
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Background and aims: Nesfatin-1 is a recently discovered regulatory peptide with anorexigenic properties. The peptide is the N-terminal part of nucleobindin 2 (NUCB2) and is highly expressed in brain areas known to regulate feeding behaviour. Outside the brain, nesfatin-1 expression has been reported in gastric endocrine cells. We studied the possibility of nesfatin-1 expression in human, rat, and mouse islets of Langerhans using immunocytochemistry and in situ hybridization. Furthermore, we investigated potential influence of nesfatin-1 on secretion of insulin and glucagon. Effects of nesfatin-1 on Ca2+ and cAMP levels was studied in INS-1 (832/32) cells, and nesfatin-1 gene expression in human islets under glucolipotoxic conditions was examined using affymetrix.
Materials and methods: Nesfatin-1 expression was examined using immunocytochemistry, in situ hybridization. Insulin and glucagon secretion after 1h static incubations of isolated mouse islets was analyzed with RIA or ELISA. Ca2+ fluorescence and cAMP was studied in INS-1 (832/13) cells.
Results: Nesfatin-1 was found to be highly expressed in human islets. Double staining for nesfatin-1 and the main islet hormones revealed that nesfatin-1 was exclusively expressed in human beta cells. Nesfatin-1 was also highly expressed in rat and mouse islets. The majority of all nesfatin-1 expressing cells were beta cells in rat and mouse. In the rat a minor subpopulation of the alpha cells harbored also nesfatin-1. Importantly, in situ hybridization and microarray for NUCB2 mRNA confirmed the immunocytochemical data. Studies in isolated mouse islets revealed that nesfatin-1 stimulates insulin secretion at both low (2.8 mM and 5.5mM, $p<0.001$) and high glucose concentrations (11.1. mM, $p<0.001$). Nesfatin-1 also augmented forskolin-enhanced glucose-stimulated insulin secretion (GSIS) ($p<0.01$), but not GSIS potentiated by carbachol or KCl. In addition, nesfatin-1 lowered secretion of glucagon at low (2.8 and 5.5 mM) glucose ($p<0.001$), but not at high (11.1 mM) glucose. Further, nesfatin-1 caused an increase in Ca2+ influx, but decreased cAMP in INS-1 (832/13) cells. Moreover, affymetric analysis revealed that NUCB2 mRNA was moderately upregulated under glucolipotoxic conditions in human islets.
Conclusion: Together our data suggest that nesfatin-1 is a novel glucose-lowering peptide in human and rodent islets. In view of these data, stimulation of the nesfatin-1 pathway could be a new treatment strategy for Type 2 diabetes.
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Expression of Bombesin Receptor Subtype-3 (BRS-3) in islets from different species and its role in the regulation of insulin secretion and glucose homeostasis
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Background and aims: Bombesin-like peptides, such as bombesin and gastrin-releasing peptide, are known to promote insulin secretion and possibly β-cell proliferation. The biological functions of these peptides are mediated by a family of G-protein coupled receptors. The expression and roles of those receptors in pancreatic islets have not been fully investigated. Here we study the physiology of Bombesin Receptor Subtype-3 (BRS-3) in islets from various species, as it has recently been shown to be involved in the regulation of energy homeostasis and BRS-3 knockout mice manifest age-dependent obesity and reduced islet size.
Materials and methods: TaqMan analysis was used to measure mRNA levels. siRNA-mediated BRS-3 silencing, whole body Brs-3 knockout and BRS-3 specific agonist or antagonist were used to study the effects of BRS-3 on insulin secretion in mouse and human islets, and glucose tolerance in mice.
Results: Utilizing quantitative PCR, we observed high levels of BRS-3 mRNA in human, dog, and mouse (but not rat) pancreatic islets. Silencing BRS-3 with siRNA or pharmacological blockade with a BRS-3 antagonist, Bantag-1, reduced glucose stimulated insulin secretion (GSIS) in the rat INS-1 832/3 cells. In contrast, activation of the receptor with Bag-1, a potent, selective BRS-3 agonist, increased GSIS in the rat insulinoma cell line. The acute effects of Bag-1 on GSIS in isolated islets and on blood glucose *in vivo* during oral glucose tolerance tests (OGTT) were examined. Bag-1 significantly enhanced GSIS in isolated islets and reduced OGTT glucose levels in wild-type, but not *Brs3* knockout mice. BRS-3 agonists also promoted GSIS in human islets isolated from a non-diabetic subject and from a patient with type 2 diabetes. These results reveal a role for BRS-3 in islet physiology, with agonism directly promoting GSIS.
Conclusion: In addition to its potential role in the regulation of body weight and energy homeostasis, BRS-3 may also regulate glucose homeostasis. Modulation of BRS-3 represents a potential new mechanism for glycemic control in type 2 diabetic patients.

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p42/44 MAPK activation is required for receptor-operated stimulation of insulin release
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Background and aims: We have previously shown that activation of the receptors GPR54 and CaR, by kisspeptin and the CaR agonist A568 respectively, potentiates glucose-induced insulin release via a p42/44 MAPK dependent pathway. The present study aimed to further examine the role of p42/44 MAPKs in receptor-operated potentiation of insulin release.
Materials and methods: Mouse islets were incubated for 1hr in a physiological salt solution and insulin release was measured by radioimmunoassay. Activation of p42/44 MAPK was measured by western blotting for phospho-MAPK relative to total MAPK immunoreactivity.
Results: Both the CaR agonist A568 (10μM) and kisspeptin (1μM) caused a significant potentiation of insulin secretion (214±26% and 182±19% basal respectively, $n=9$, $p<0.05$) at 20mM glucose. The stimulatory effects of both A568 and kisspeptin on insulin secretion were significantly blocked by the presence of the p42/44 MAPK inhibitor PD098059 (50μM; 131±12% and 109±8% basal respectively, $p<0.05$ versus absence of PD098059). However, whilst the acetyl choline analogue carbachol (500μM) also significantly potentiated glucose-induced insulin release (287±21% basal, $n=9$, $p<0.05$), the

presence of PD098059 had no effect. Furthermore, whilst activation of p42/44 MAPK was enhanced by incubation (5 min, 37°C) in the presence of stimulatory glucose levels, kisspeptin (1μM) had no effect on MAPK activation. A568 caused an increase in p42/44 MAPK activation at 2mM glucose, but had no effect at 20mM glucose. Finally islets were incubated in the presence of okadaic acid and sodium pervanadate in order to inhibit the dephosphorylation of p42/44 MAPK. Whilst neither kisspeptin nor A568 significantly affect insulin release from islets at sub-stimulatory glucose concentrations under normal conditions, in the presence of okadaic acid and sodium pervanadate (10μM and 100μM respectively) both kisspeptin and A568 caused a significant increase in insulin release from islets incubated at 2mM glucose (381±52% and 456±39% basal respectively, n=9, p<0.05).
Conclusion: These observations suggest that p42/44 MAPK activation may play a permissive role in the potentiation of glucose-induced insulin secretion by some receptor operated agonists such as kisspeptin and A568.
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***In vitro* functions of pancreatic pseudoislets**
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Background and aims: Recently it was shown, that transplantation of small islets results in a better outcome than transplantation of large islets. Small islets can be produced in vitro by dissociating islets of all sizes in single cells and reaggregating the dispersed islet cells into pseudoislets of defined small sizes by the hanging drop method. We describe an alternative method to produce pseudoislets of different sizes in micro-well plates and analyse their morphology and in vitro function.
Materials and methods: Islets were dissociated into single cells by trypsin/EDTA treatment. The cells are seeded at defined densities into hanging-drops (HD) or especially designed micro-well plates. Plates of four different types of plastics were used: two types of polycarbonate (PC and NO), one polystyrene (PS) and one cyclic olefin copolymer (TO). Islets and seeded islet cells were cultivated for 7-14days and the resulting pseudoislets analysed in terms of morphology and glucose-stimulated-insulin-secretion (GSIS).
Results: Dissociated islet cells reaggregated to pseudoislets in micro-well plates. There was no significant difference in pseudoislet size between the different plastics. Pseudoislets originating from 300 seeded cells have an average diameter of (in micrometer) NO 60.9±15.4 n=15, PC 62.7±20.7 n=14, PS 70.7±17.7 n=9, TO 65.4±14.1 n=18; NO-PS p=0.19. The diameter of pseudoislets is approximately proportional to the numbers of cells seeded (to the power of 0.33), for NO with 600 cells 76.4±17.2 n=6. Pseudoislets from micro-well plates are smaller and have a larger variation in diameter as compared to HD-pseudoislets with 300 cells 93.1±6.1 n=4, HD with 750 cells 135.5±4.9 n=4, with 1500 cells 180.6±5.0 n=4. GSIS was similar for islets from micro-well plates and HD (basal 0.1 - 0.2, stimulated 1.2 - 1.5 fmol insulin/IEQ/min). In contrast to HD-pseudoislets derived from rat islets, GSIS was not increased in human pseudoislets (HD or micro-well plates). Human pseudoislets however exhibited an improved first phase insulin secretion (300cells per pseudoislet 1.94±0.5 fold steady state second phase, n=32; 600 cells per pseudoislet 1.95±0.15 n=5) compared to intact islets (1.31±0.33 n=57; p<0.005) whereas in rat pseudo- and intact islets there was no clear first phase insulin secretion.
Conclusion: The newly developed micro-well plates offer the possibility to produce large numbers of pseudoislets of similar quality as compared to the hanging-drop method. Small human pseudoislets exhibit an equal or improved insulin secretion as compared to intact islets. The true advantage of small pseudoislets in transplantation due to smaller size and better diffusion properties has to be proven in a large animal model.
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Pharmacological effects on insulin release in a mathematical model of human beta cells
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Background and aims: Beta-cells use electrical activity to transduce changes in plasma glucose to calcium-triggered exocytosis and insulin secretion. Using a mathematical model based on recent electrophysiological characterizations of human beta-cells, the effect of a range of pharmacological interventions on patterns of electrical activity and exocytosis was investigated.
Materials and methods: A mathematical model based on high quality data from human beta-cells has been developed. The model is formulated as a set of ordinary differential equations, and describes voltage-gated K- and Na-channels, as well as T-, P/Q- and L-type Ca-channels. In addition, a background leak current, and ATP-sensitive K(ATP) and Ca-activated BK-potassium channels are also included. Exocytosis evoked by calcium currents through different types of Ca-channels is modeled. Numerical simulations corresponding to pharmacological interventions were performed.
Results: The model reproduces satisfactorily electrical patterns in response to blockage of various ion channels. The central role of K(ATP)-channels is shown, and electrical activity is a result of K(ATP)-channel closing by glucose or tolbutamide. For K(ATP)-conductance larger than ~0.02 nS/pF the model is silent and hyperpolarized, while it shows spiking activity for smaller K(ATP)-conductances, which results in exocytosis. A role for sodium and calcium channel activation for the upstroke, and activation of potassium channels for the downstroke of action potentials is found by simulating ion channels blockage. In the presence of K-channel blockers such as TEA, inactivation of Ca-channels is responsible for repolarization. It is shown that P/Q-types calcium currents are crucial for evoked exocytosis, and that the result on insulin secretion of modification of other channels is transduced mainly by changes in electrical activity and P/Q-type Ca-currents. Modifying a leak current is shown to be a possible strategy for enhancing insulin secretion.
Conclusion: A mathematical model of electrical activity in human beta-cells is shown to predict the effects of ion channel modulating drugs. It is shown to be useful for hypothesis testing and prediction of the effect of ion channel modulation on electrical activity and insulin secretion.
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Developing a mathematical model of the mechanism by which alanine enhances glucose-stimulated insulin secretion
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Background and aims: Pancreatic beta-cells play a key role in the glucose homeostasis, secreting insulin in response to blood nutrient fluctuations. This is due to a complex relationship between metabolism and insulin secretion which comprises both triggering (ATP and Ca^{2+} dependent) and amplifying (mitochondrial metabolite dependent) pathways of insulin secretion. Specific amino acids can modulate beta-cells function and thus acutely and chronically regulate insulin secretion through different mechanisms of action. Alanine is known to acutely stimulate insulin secretion alone or synergistically enhance glucose-stimulated insulin secretion (GSIS) both *in vivo* and *in vitro*. This study has attempted to develop a mathematical model, validated against wet lab experimental results, of the role played by the amino acid alanine in enhancing glucose-stimulated insulin secretion in pancreatic beta-cells.
Materials and methods: A simplified kinetic model of the glucose-stimulated insulin secretion in pancreatic beta-cells which takes into account glycolysis, Krebs cycle, NADH shuttles and glutamate and alanine transaminase was built. The model's input is made up of two nutrient components: glucose and alanine, while the output is constituted by NADH, ATP and glutamate. Experimental work was carried out on a functional clonal insulin-secreting cell line (BRIN- BD11). BRIN-BD11 cells were cultured for 48h prior to experiments, starved for 40 minutes at basal glucose level (1.1 mM) and then stimulated with different concentrations of only glucose (1.1, 5, 16.7, 30 mM), only alanine (10 mM) and their combinations. Samples were collected before and 10, 20, 60 minutes after administration of the stimulus. Samples were assayed for consumption and production of the key components of the mathematical model: glucose, lactate, ATP and insulin.
Results: Both glucose consumption and lactate production showed a dose-dependent increase which was significantly enhanced by addition of 10 mM alanine (p<0.001 and p<0.05, respectively at both 16.7 mM and 30 mM glucose). The kinetic of ATP production following stimulation over a 60 minutes period revealed that different stimulatory conditions can affect not only the magnitude of response, but also the kinetic mechanism of release. All stimulatory conditions showed an increase in ATP release over the first 20 minutes, subsequently ATP content reached a steady state and markedly decreased after 60 min incubation only in presence of alanine. Acute insulin secretion

was biphasic and concentration dependent with respect to glucose: increasing glucose concentration from 1.1 to 30 mM increased insulin secretion by 32% from 1.12 ng/mg protein/20 min to 1.48 ng/mg protein/20min. The addition of 10 mM alanine significantly increased ($p<0.05$) glucose stimulated insulin secretion by 1.8-2.3 fold after 20 minutes incubation. Administration of 10 mM alanine exhibited a strong insulin release (1.39 ng/mg protein/ 20 min).
Conclusion: These results highlight the synergistic effect of Alanine in enhancing glucose metabolism. Alanine metabolism may increase ATP release increasing insulin secretion through the K^+ ATP-dependent pathway. Alanine oxidation may also result in generation of glutamate, a putative messenger in insulin secretion, which may account for increased insulin release despite a lower ATP content.
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The membrane potential response is altered in pancreatic beta cells chronically exposed to high glucose

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Background and aims: Fuel-stimulated insulin secretion is coupled to increased cellular metabolic activity and consequently an increase of the ATP/ADP ratio, thereby leading to plasma membrane depolarisation and exocytosis. Any disturbance of the metabolic processing of fuels, such as glucose, particularly in mitochondria, may consequently affect the secretory process. Frequent episodes of severe hyperglycaemia are observed during the onset of diabetes and characterise poorly- or un-controlled Type 2 Diabetes. These elevated glucose levels lead to beta-cell desensitisation, and ultimately impaired beta-cell function. It is therefore of great interest to detect potential disturbances of normal fuel-stimulated plasma membrane changes as a result of chronic exposure to elevated glucose levels.
Materials and methods: INS-1 832/13 cells were cultured in either 2.8 mM or 16.7 mM glucose for 48 h. All cells were then starved for 2 h in buffer containing 2.8 mM glucose, after which recordings of the plasma membrane potential of a monolayer of cells, using a proprietary fluorescent anion and confocal microscopy were performed at 16.7 mM glucose with subsequent additions of 1 μg/ml oligomycin and 25 mM KCl. Insulin secretion was determined by radioimmunoassay.
Results: After 48 h culture in 16.7 mM glucose, glucose-stimulated insulin secretion was significantly ($p<0.05$) suppressed in INS-1 832/13 cells (15.32 ± 1.6 ng/mg/h) in comparison to cells cultured at 2.8 mM glucose (41.43 ± 8.13 ng/mg/h). Concurring, chronic high glucose levels altered the plasma membrane depolarisation response. Recordings of membrane potential activities of cells cultured at 2.8 mM glucose for 48 h showed complete hyperpolarisation before glucose stimulation (-79.5 ± 1.6 mV). Following addition of 16.7 mM glucose a mean depolarisation of all cells measured was recorded (-58.1 ± 0.4 mV) and individual cells produced rhythmic action potentials bursts, acquired in real time as oscillations in fluorescence intensity. By inhibiting the mitochondrial ATP synthase, oligomycin hyperpolarised the plasma membrane potential to basal values while high KCl produced a maximum membrane depolarisation of -41.6 ± 0.1 mV. In contrast, cells chronically cultured at 16.7 mM glucose produced rhythmic bursts of action potentials after the starvation period even before high glucose stimulation. The basal membrane potential recorded was higher (-72.1 ± 0.6 mV) than in cells cultured at 2.8 mM glucose. In addition, the mean depolarisation response of the chronic high glucose-treated cells was suppressed (-62.7 ± 0.3 mV) after anew stimulation with high glucose.
Conclusion: While cells kept at low glucose responded to stimulatory glucose concentrations with elevated plasma membrane depolarisation and stimulation-induced action potential bursts, in cells under chronic high glucose conditions this depolarisation response was greatly diminished. However, action potential bursts were recorded independent of glucose stimulation due to the inability of glucose-desensitised cells to fully hyperpolarise. We suggest that, as a consequence of chronic fuel overload, multiple cellular alterations, including changed glucose metabolism, contribute to a disturbance in the fuel-dependent coordination and regulation of plasma membrane potential changes and therefore the failure of beta-cells to secrete insulin.

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Paradoxical membrane repolarisation during onset of insulin secretion

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Background and aims: Plasma membrane depolarization (typically by K_{ATP} channel closure), activation of voltage-dependent Ca^{2+} channels and Ca^{2+} influx are regarded as indispensable for the initiation of stimulated insulin secretion. In addition to this "triggering pathway" nutrient secretagogues like glucose or ketoisocaproic acid (KIC) activate an "amplifying pathway" not involving depolarization and Ca^{2+} influx. The metabolic amplification can be demonstrated by a pretreatment with a maximal sulfonylurea concentration, then adding a stimulatory concentration of a nutrient secretagogue. Surpris-

ingly, we found the addition of nutrients to be related to a repolarization, thus abolishing the triggering signal which is believed to be necessary for the nutrient amplification to exert its effect.
Materials and methods: The plasma membrane potential of normal mouse beta cells was measured in the perforated patch mode of patch-clamp technique. The cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) as indicated by Fura and the NAD(P)H autofluorescence was measured by microfluorimetry of single perifused islets. NAD(P)H autofluorescence lifetime was determined by 2 photon excitation imaging. Insulin secretion was measured by batch perifusion and ELISA of the fractionated effluate.
Results: In the absence of any nutrient 2.7 μM glipizide depolarized the plasma membrane to a plateau of -45.5 ± 3.0 mV with superimposed action potentials peaking at -24.4 ± 4.0 mV (n=5). After application of 30 mM glucose or 10 mM KIC the membrane completely repolarized ($ -68.4 ± 2.1 mV) within 150 s (glucose) or 80 s (KIC). The repolarization phase lasted for 5.5 ± 2.0 minutes (glucose) and 6.8 ± 2.9 minutes (KIC) and then abruptly, the depolarization reappeared with the same characteristics. Concurrent with the depolarization by K_{ATP} channel closure, glipizide increased the $[Ca^{2+}]_i$, which remained elevated until the addition of nutrients. Both with glucose and with KIC the repolarization coincided with a biphasic decrease of the $[Ca^{2+}]_i$ down to values which existed before the exposure to glipizide (n = 4 each). While the elevated $[Ca^{2+}]_i$ completely recovered within 10 minutes in the case of glucose, there was only a partial recovery of $[Ca^{2+}]_i$ in the presence of KIC. Under the same conditions 10 mM KIC induced a strong increase of the secretion rate (by more than 10fold within 10 min), which remained elevated for more than 30 min. In contrast, 30 mM glucose was unable to further elevate the secretion rate established by glipizide in the absence of nutrients. Both glucose and KIC elevated the NAD(P)H autofluorescence of perifused islets, the relation between the increase by glucose and that by KIC was the same as in the absence of glipizide. Since both components of NAD(P)H fluorescence lifetime were left unchanged, both glucose and KIC induce a real increase of the mass of NAD(P)H and not a change in fluorescence properties.
Conclusion: Apparently glucose and KIC differ in their mechanisms of metabolic amplification. The onset of a strong and lasting secretory response to KIC does not necessarily require the presence of a depolarized plasma membrane and an increased $[Ca^{2+}]_i$.

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In situ electrophysiological examination of alpha cells in type 1 diabetes revealing the cellular basis of glucagon hypersecretion
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Background and aims: The cellular properties of α cells in type 1 diabetes (T1D) are unknown. This is because T1D autoimmune destruction of β cells causes the islet mass to shrink in size rendering islet isolation and dispersion not technically feasible; and consequently electrophysiological characterization of islet cells to reveal the underling mechanisms explaining the distorted glucagon secretion in T1D could not be done. We employed GluCre-ROSA26-YFP (GYY) mice, which expresses YFP in pancreatic α cells. Along with our newly developed pancreas slice preparation whereby α cell and its precise secretory physiology within intact pancreatic tissue can be examined by patch clamp technique, unperturbed by conventional islet isolation and dispersion procedures, we are able to reliably localize and directly examine α cells electrophysiological properties *in situ* in health and T1D. We hypothesize that T1D α cells possess perturbed ion channels properties which contribute to hyperglucagonemia in early stage of T1D.
Materials and methods: GYY mice were treated with streptozotocin (STZ) to induce T1D. IPGTT and radioimmunoassay (RIA) were performed to confirm diabetes phenotype. Pancreas slices were prepared from these mice to directly examine α cells ion channel properties in healthy and diseased islets by patch clamp technique. The identities of patched-cells were further confirmed by infusing fluoresecent marker (biocytin) during patching, showing its co-localization with YFP by confocal microscopy.
Results: Normal GYY mice α cells in slices revealed identical electrophysiological features to those of their background C57/BL6 mice we previously characterized. These α cells are equipped with readily-activated A-type I_K, voltage-gated I_{Na}, small size, low resting conductance, and inducible H/LVA I_{Ca} at -80mV. I_{Ca} influx correlated with glucagon exocytosis as either train of depolarization or UV photo-release of intracellular-loaded caged-Ca^{2+} stimulated C_m increase. 4 weeks after STZ treatment, GYY mice developed T1D, exhibiting higher fasting glucose, slower glucose clearance after a glucose challenge and higher fasting (control; 89 pg/ml vs. STZ group;122 pg/ml) and fed (control; 78 pg/ml vs. STZ group;112 pg/ml) serum glucagon levels. α cells in slices from these diabetic mice revealed augmentation of I_{Na} (control; 368 ± 43 pA vs. STZ group; 480 ± 71pA) and LVA I_{Ca} amplitudes (control; 40 ± 5 vs. STZ group; 49 ± 6 pA). HVA I_{Ca} however remained unaltered by T1D (control; 44 ± 5 pA vs. STZ group; 46 ± 6 pA) . Voltage-gated K^+ current was found to be increased (STZ group; 2.13 nA vs. control; 1.76 nA). α cell size was unchanged compared to control (control; 4.7 ± 0.20 pF vs. STZ group; 4.8 ± 0.23 pF).
Conclusion: GYY mouse α cell ion channel properties examined in slices were largely consistent with our previous findings and others, validating the feasibility of using pancreas slice approach to investigate α cells in normal and diabetic subjects. We postulate that the observed upregulation of I_{Na} and LVA I_{Ca} in diabetic α cells potentially elevates membrane potential that would more readily to trigger HVA Ca^{2+} channels opening, with ensuing initiation of action potential firing leading to glucagon secretion. This explains in part the observed glucagon hypersecretion in early stage of T1D.

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Active CFTR channels are important for insulin- and glucagon secretion
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Background and aims: Cystic fibrosis (CF) is a monogenic autosomal recessive disease caused by mutation in the cystic fibrosis gene that encodes the chloride channel cystic fibrosis transmembrane conductance regulator (CFTR). One of the leading complications of CF is cystic fibrosis related diabetes (CFRD). The mechanism behind CFRD is unclear but the progressive inflammation in the exocrine pancreas has been suggested. Another possible mechanism is that CFTR is present in the islet cells and a mutation in CFTR interferes with hormonal secretion. The aim of this study was to investigate the presence of active CFTR channels in pancreatic alpha- and beta cells and if CFTR influence secretion and exocytosis.
Materials and methods: Patch-clamp recordings and capacitance measurements were performed on single mouse beta-cells. Detection of CFTR in islet cells was investigated using rt-PCR and confocal immunocytochemistry. Insulin-and glucagon secretion was measured using radio immunoassay (RIA).
Results: Mouse islets express CFTR mRNA as confirmed by rt-PCR (*n*=5) and CFTR protein was specifically detected in alpha- and beta cells using immunocytochemistry. Electrophysiological investigation of CFTR was performed using the standard whole-cell configuration and CFTR was activated by addition of forskolin (10 μM). In single mouse beta-cells, a cAMP-activated membrane conductance of 0.05 ± 0.03 nS/pF at negative potentials and 1.03 ± 0.18 nS/pF at positive potentials (*n*=12; P<0.001 *vs* in absence of forskolin) was measured. The conductance was significantly reduced (*n*=7; P<0.001) and the current almost totally inhibited in the presence of the CFTR-antagonist, CFTRinh-172 (10 μM). A similar CFTR current could be activated in pancreatic alpha-cells (*n*=5; P<0,017). In addition, cAMP-amplified glucagon secretion measured at 1 mM glucose was reduced by ~60% (*n*=8; P<0.001) in the presence of CFTRinh-172 (40 μM) and by ~40% (*n*=8; P<0.08) in the presence of another CFTR-antagonist, GlyH-101 (50 μM). Cyclic AMP- amplified insulin secretion at 16.7 mM glucose was reduced by ~30% in the presence of CFTRinh-172 (*n*=10; P<0.05) and by ~30% in the presence of GlyH-101 (*n*=11; P<0.05). Moreover, exocytosis elicited by a train of 10 membrane depolarisations and measured as an increase in membrane capacitance on single beta-cells was significantly reduced by 70 ± 10% (*n*=9; P<0.05) in the presence of cAMP (100 μM) and CFTRinh-172 (10 μM).
Conclusion: Our data indicate the presence of active CFTR in pancreatic alpha- and beta cells and the importance of this channel for glucagon- and insulin secretion. Further, CFTR inhibition reduced exocytosis in pancreatic cells. Thus, we suggest a role for CFTR in the control of the exocytotic process important for release of glucagon and insulin.
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Distinct roles of voltage-gated potassium channels Kv2.1 and Kv2.2 in governing the secretion islet hormones

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Background and aims: Voltage-gated potassium channel Kv2.1 has been suggested to regulate glucose stimulated insulin secretion (GSIS) in islets, yet little is known about the role of Kv2.2, the other member of the delayed rectifier K channel also expressed in islets.
Materials and methods: We studied the roles played by each of the two Kv channels individually in islets using siRNA mediated gene-silence, Kv2.1 knockout (Kv2.1-/-) mice and highly selective peptide and small molecule Kv2 blockers newly discovery in our laboratories.
Results: siRNA directed against Kv2.1 in INS-1 cells remarkably reduced the Kv current and augmented GDIS, whereas Kv2.2 siRNA had no effect on GDIS. Pancreatic β-cells from Kv2.1 knockout mice manifested significantly smaller Kv current and greater GDIS *in vitro*. GxTx-1E diminished Kv current completely and enhanced GDIS dramatically in isolated mouse islets. But surprisingly, GxTX-1E only had a minimal *in vivo* effect to lower blood glucose levels during IPGTT (intraperitoneal glucose tolerance test) in mice. We thus examined the effects of GxTx-1E and Kv2i-A, a small molecule blocker of Kv2.1 and Kv2.2 discovered in our laboratories, on the release of glucagon and somatostatin, the other two major islet hormones. Besides promoting insulin secretion, both GxTX-1E and Kv2i-A enhanced somatostatin (SST) release from the cultured islets or *in vivo* animals. The effect of GxTX-1E to promote SST secretion was still seen in islets from Kv2.1 knockout mice, and was further augmented when Kv2.2 expression was 65% ablated by adenoviral delivered siRNA, indicating that Kv2.2 but not Kv2.1 regulates SST secretion in the pancreatic islets. This was corroborated by high expression of Kv2.2 in SST secreting δ-cells of the islets revealed by *in situ* hybridization and immunohistochemistry.
Conclusion: Kv2.1 and Kv2.2 have distinct roles in regulating islet insulin and SST secretion. Development of selective Kv2.1 inhibitor and Kv2.2 activator may provide new avenue for novel insulin secretagogues for diabetes therapy.

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Evidence of functional hemi-channels in beta cells: their opening by K^+-depolarisation and/or Ca^{2+}-omission

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Background and aims: We have previously reported that islet depolarization (70 mM KCl + 0.25 mM diazoxide) induces release of GABA and taurine (Tau) and suppresses KIC-induced insulin secretion. The aim of this work is to characterize the responsible mechanism of this increased amino acid release.
Materials and methods: Insulin secretion of rat perifused islets was monitored by RIA. Islet content and release of amino acids were measured by fluorescent detection after pre-column derivatization with o-phtalaldialdehyde and their HPLC separation. Islet ATP and ADP contents were measured with the luciferin/luciferase system.
Results: At 5 mM glucose, Ca^{2+}-omission (0 mM Ca^{2+} + 0.1 mM EGTA) stimulated islet release of GABA (438.5 ± 35.4, n=4 vs.289.7 ± 41.2 pmol/30 islets x 60 min, n=4; p<0.03) and Tau (119.3 ± 11.5, n=4 vs. 66.7 ± 4.2 pmol/30 islets x 60 min, n=4; p<0.005) and suppressed proportionately their contents. 70 mM KCl depolarization potentiated the releasing effect of Ca^{2+}-omission on both GABA (747.1 ± 98.7, n=4 vs. 438.5 ± 35.4 pmol/30 islets x 60 min, n=4; p<0.03) and Tau (194.1 ± 28.1, n=4 vs. 119.3 ± 11.5, n=4 pmol/30 islets x 60 min, n=4; p<0.05) and diminished proportionately their contents. Islet ATP release could not be measured due to the presence of a very efficient ecto-nucleotidase activity resistant to available inhibitors. However, at 3 mM glucose, ATP content was decreased by the combination of depolarization and Ca^{2+}-omission (1.3 ± 0.14, n=12 vs. 2.8 ± 0.14 pmol/ islet, n=18; p<0.0001) without affecting the ATP/ADP ratio (p=0.05). At physiological $[Ca^{2+}]_o$, islet depolarization alone suppressed ATP content at 3 mM glucose (0.9 ± 0.1, n=6 vs. 2.9 ± 0.3 pmol/islet, n=6; p<0.0001) and slightly decreased the ATP/ADP ratio (0.95 ± 0.05, n=5 vs. 1.26 ± 0.08 pmol/ islet, n=5; p<0.011). 20 mM glucose prevented the loss of islet ATP induced by the combination of depolarization and Ca^{2+}-omission observed at 3 mM glucose (4.4 ± 0.16, n=8 vs. 4.6 ± 0.09 pmol/islet, n= 12; N.S.) and preserved the elevated ATP/ADP ratio (1.8 ± 0.17, n=7 vs. 2.2 ± 0.22 pmol/islet, n=12; N.S.). Mefloquine (50 uM) did not modify islet ATP content at 3 mM glucose but counteracted the combined effects of islet depolarization + Ca^{2+}-omission (2.1 ± 0.27, n=8 vs. 1.3 ± 0.14, n=12 pmol/islet; p<0.006); it also partially suppressed islet amino acid release under the same experimental conditions. 20 mM glucose stimulation of insulin secretion in perifused islets (100 ± 9.3%) was dose-dependently decreased by mefloquine to 84.8 ± 11.5% (10 uM; N.S.), 53.7 ± 7.3 % (25 uM; p<0.01) and 27.5 ± 5.2 % (50 uM; p<0.001). At concentrations above 100 uM, it stimulated a secretory response that was independent of the glucose and calcium concentration and it decreased islet insulin content.
Conclusion: Islet amino acids and ATP are released by procedures known to open hemi-channels/pannexins in other tissues. This is supported by the protective effect of mefloquine, a known connexin 36 inhibitor. Inhibition of insulin secretion by mefloquine highlights the importance of beta cell communication for an optimal insulin response to glucose.
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Analysis of submembrane insulin granule behaviour by TIRF microscopy during moderate and strong K^+ depolarization

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Background and aims: The release of a pool of membrane-adjacent secretory granules which are in a primed and docked state and await one final trigger, a depolarization-induced influx of Ca^{2+}, is held responsible for the first phase of glucose-induced insulin secretion. Recently, we observed that 15 mM K^+ led to a 20 mV depolarization and to a lasting increase of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$), but only to a modest transient increase in secretion, suggesting that the effect of depolarization on insulin secretion is only incompletely understood.
Materials and methods: Insulin secretion was measured by perifusion of mouse islets and MIN6 pseudoislets and ELISA of the fractionated effluate. The $[Ca^{2+}]_i$ of islets and MIN6 cells was measured with the Fura technique. Submembrane granules were visualized by transient transfection of MIN6 cells with an insulin-EGFP fusion protein and imaging by TIRF microscopy at 37°C. The images were evaluated by a purpose-made program written in MatLab to achieve a complete observer-independent quantitation.
Results: Islets perifused with 5 mM glucose showed only a transient increase in secretion when K^+ was raised to 15 mM. A subsequent elevation to 40 mM K^+ resulted in a prompt overshooting increase in secretion which remained increased as long as 40 mM K^+ was present. In contrast, $[Ca^{2+}]_i$ was continuously elevated by 15 mM K^+ and increased further when K^+ was raised to 40 mM. The same secretion and $[Ca^{2+}]_i$ pattern could be observed with MIN6 pseudoislets. MIN6 cells transfected with insulin-EGFP were therefore used to analyse the behavior of submembrane granules by TIRF microscopy at 5 time points: beginning, prior to high K^+, one each during 15 and 40 mM K^+ and one after washout of high K^+. At each time point a sequence of 100 images was acquired within 12 s. Referring to the first sequence as 100%, the numbers of submembrane granules at the next time points were 109%, 76%, 50% and 56%. More than 50% of the granules were long-term resident (≥ 12 s). The percentage of short-term resident granules (≤ 1 s) increased slightly during 15 mM K^+, then significantly during 40 mM K^+ and remained so after washout. This correlated with an increase of the percentage of newly arriving granules from 4% initially to 7% during 15 mM K^+ and to 15% during 40 mM K^+ and washout. Very similar percentages applied for departing granules (i.e. return or release). There were no such changes in control experiments. The total and the net distances covered by the submembrane granules were not affected by high K^+.
Conclusion: 40 mM K^+ affects arrival, departure and residence time of insulin granules, but not movement parallel to the membrane. 15 mM K^+ is moderately effective, which concurs with dynamic secretion measurements. Currently there is no indication that long-term resident granules are preferentially departing during stimulation.

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Abnormal regulation of pancreatic beta cell Na,K-ATPase on glucose intolerant rats

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Background and aims: Glucose (G) is the most important physiological insulin secretagogue. It is widely accepted that, in pancreatic -cell, G evoked early ionic events such as membrane depolarization and Ca^{2+} influx through voltage dependent Ca^{2+} channels triggers insulin exocytosis. However, the role of other electrogenic systems, namely ionic pumps, to these events remains essentially uninvestigated. It is known that the activity of Na,K-ATPase is modified in type 2 diabetes (T2D). The pump is responsible for maintaining Na^+ and K^+ gradients across the plasma membrane and generates a net outward current as a result of $3Na^+/2K^+$ exchange. It remains elusive whether Na,K-ATPase activity is regulated by G in pancreatic β-cell and/or this current contributes to the ionic events regulating insulin secretion. The aim of this work was to assess G evoked regulation of Na,K-ATPase activity in intact -cells of normal and G intolerant rats.
Materials and methods: Pancreatic -cells, from normal (controls) or glucose-intolerant Wistar rats (GIR), were isolated and cultured (48h). Cell batches were pre-incubated (30min) with 2.1mM G to reach basal. Afterwards cells were challenged with [G] in the interval 0-11.1mM for 60min, for dose-dependence evaluation, or with 8.4mM G for 5-120min, for time-dependence evaluation. ATPase activity was assessed in intact cells by colorimetric quantification of Pi formed in 30min. Na,K-ATPase activity was calculated by the difference between the activities obtained in the absence and in presence the of 1mM ouabain.
Results: G evoked both time- and dose-dependent regulation of Na,K-ATPase. In β-cells from controls, G induced a bimodal regulation of Na,K-ATPase. In the absence of G, Na,K-ATPase activity was 0.056±0.015U/mg. Raising [G] to 2.1mM induced a ≈3 fold increase of Na,K-ATPase activity whereas a further increase in [G] in the interval of 5.6-11.1mM evoked a significant reduction of Na,K-ATPase activity to the levels observed in the absence of the secretagogue. Compared to 2mM G, the activity was reduced in 68%, 55% and 66% when [G] was increased to 5.6, 8.4 and 11.1mM, respectively (n=3-12). GIR β-cells exhibit an altered profile of response to the secretagogue; In the absence of G, Na,K-ATPase activity was ≈4 fold the activity observed in the controls (0.202±0.036U/mg; n=3). The pump activity remained unchanged for 2.1-5.6mM G and similar to maximal activity observed in the controls (0.188±0.035 U/mg, for 2.1mM G; n=4). A significant reduction of the pump activity in GIR β-cells was induced by 8.4mM G (0.118±0.018 U/mg). G (8mM) induced a time-dependent inhibition of Na,K-ATPase with a biphasic profile. Pump activity decreased to a minimum value (32%) after 20min exposure to G, showing a partial recovery to 45%, 46% and 47% for 30, 60 and 120min, respectively (n=5-12). GIR β-cells showed an attenuated response to G (59% activity after 20min) without any recovery (n=5-11).
Conclusion: This work demonstrates that Na,K-ATPase is finely regulated by G in pancreatic β-cell from normal subjects. This regulation is impaired in GIR where desensitization and an attenuation of the inhibitory action of G were observed. In summary, Na,K-ATPase contribution to G-induced ionic events and insulin secretion might be relevant in T2D development.
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PS 38 Ca^{2+} and cAMP in beta cells

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Interplay between [Ca2+]c and [Ca2+]ER in mouse beta cells: role of SERCA2b and SERCA3

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Background and aims: Pancreatic β-cells express 2 types of sarco-endoplasmic Ca^{2+}-ATPases, SERCA2b and SERCA3, which take up Ca^{2+} from the cytosol to the endoplasmic reticulum (ER). Whereas the changes in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) have been well characterized in β-cells, the changes in the ER Ca^{2+} concentration ($[Ca^{2+}]_{ER}$) are still largely unknown. Here, we studied the correlation between $[Ca^{2+}]_c$ and $[Ca^{2+}]_{ER}$ and the roles of SERCA2b and SERCA3 in $[Ca^{2+}]_{ER}$ homeostasis.
Materials and methods: We generated an adenovirus encoding the Ca^{2+} indicator D4 and addressed it to the endoplasmic reticulum (D4ER). D4ER was expressed under the control of the rat insulin promoter in clusters of β-cells from C57BL6 (WT) mice or SERCA3KO mice. In most experiments, $[Ca^{2+}]_{ER}$ (D4ER) and $[Ca^{2+}]_c$ (Fura PE3) were simultaneously recorded.
Results: Confocal microscopy and immunocytochemistry demonstrated that D4ER was specifically expressed in the ER of β-cells. We ascertained our ability to study the correlation between $[Ca^{2+}]_c$ and $[Ca^{2+}]_{ER}$. This was the case since 45mM KCl increased both $[Ca^{2+}]_c$ and $[Ca^{2+}]_{ER}$, whereas acetylcholine elicited the expected anti-parallel changes of both parameters in β-cells from WT mice. During spontaneous $[Ca^{2+}]_c$ oscillations induced by 15mM glucose (G), $[Ca^{2+}]_c$ and $[Ca^{2+}]_{ER}$ oscillated in phase. G-induced $[Ca^{2+}]_c$ oscillations were larger and much steeper, whereas $[Ca^{2+}]_{ER}$ oscillations were smaller in SERCA3KO than in WT mice, suggesting that G-induced $[Ca^{2+}]_{ER}$ oscillations partly involve SERCA3. We then evaluated the relative contribution of SERCA2b or SERCA3 to the refilling of the ER in Ca^{2+} elicited by an acceleration of cell metabolism. In the continuous presence of diazoxide, i.e. when $[Ca^{2+}]_c$ remained low, G dose-dependently increased $[Ca^{2+}]_{ER}$ (half-maximal and maximal effects at 5 and 8 mM, respectively) and to a similar extent in β-cells from WT and SERCA3KO mice, demonstrating that SERCA2b is the only isoform responsible for this replenishment. To evaluate the contribution of both SERCA isoforms to the refilling of the ER in Ca^{2+} triggered by a rise in $[Ca^{2+}]_c$, β-cells were submitted to depolarizations with various [KCl] (10, 15, 25, 35, 45mM). As expected the rise in $[Ca^{2+}]_{ER}$ increased dose-dependently with the depolarizations. In WT β-cells, the changes in $[Ca^{2+}]_c$ and $[Ca^{2+}]_{ER}$ were parallel soon after the depolarization. Intriguingly, when $[Ca^{2+}]_c$ was kept high for a prolonged period (> 2 min with [KCl] ≥ 25mM), $[Ca^{2+}]_c$ and $[Ca^{2+}]_{ER}$ changes were antiparallel demonstrating that the ER started to release Ca^{2+} by a process that is referred to as atypical Ca^{2+}-induced Ca^{2+} release (CICR). The rises in KCl-elicited $[Ca^{2+}]_c$ and $[Ca^{2+}]_{ER}$ were, respectively, larger and smaller in SERCA3KO than in WT β-cells demonstrating that SERCA3 contributes to the refilling of the ER in Ca^{2+} when $[Ca^{2+}]_c$ increases. No atypical CICR was observed in β-cells from SERCA3KO mice.
Conclusion: $[Ca^{2+}]_c$ and $[Ca^{2+}]_{ER}$ oscillate in phase during spontaneous $[Ca^{2+}]_c$ oscillations induced by G. SERCA2b is the only isoform responsible for the Ca^{2+} replenishment of the ER elicited by an acceleration of cell metabolism whereas SERCA3 also contributes to the Ca^{2+} refilling of the ER when $[Ca^{2+}]_c$ increases. During prolonged and prominent $[Ca^{2+}]_c$ elevation, the ER releases Ca^{2+} possibly to avoid its overfilling by SERCA3.

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Spatial control of Epac2 by cAMP and Ca^{2+}

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Background and aims: Glucose-stimulated insulin release from β-cells is pulsatile and controlled by synchronized oscillations of the Ca^{2+} and cAMP concentrations beneath the plasma membrane ($[Ca^{2+}]_{pm}$ and $[cAMP]_{pm}$). Important effects of cAMP are mediated by the guanine nucleotide exchange factor Epac2, which promotes insulin secretion through activation of the small GTPase Rap1, as well as via interactions with the K_{ATP}-channel and components of the exocytosis machinery. Epac2 undergoes a conformational change upon cAMP binding allowing interaction with downstream effectors, but little is known about the subcellular localization of Epac2. The aim of the present study was to investigate the spatial control of Epac2 in β-cells by cAMP and Ca^{2+} signals.

Materials and methods: Evanescent wave fluorescence imaging was used to record plasma membrane association of fluorescence-tagged Epac2 in single MIN6 β-cells. $[cAMP]_{pm}$ and $[Ca^{2+}]_{pm}$ was measured in parallel using a fluorescent cAMP translocation biosensor and the Ca^{2+} indicator Fura Red, respectively.
Results: Rise of intracellular cAMP or direct activation of Epac by the selective agonist 007-AM caused rapid translocation of GFP- Epac2 from the cytoplasm to the plasma membrane. Increase of the glucose concentration from 3 to 11 mM triggered oscillatory translocation of GFP-Epac2 that were preceded by elevations of $[cAMP]_{pm}$ and $[Ca^{2+}]_{pm}$. The translocation was suppressed by 43±20% (n=16, P<0.01) and 41±8% (n=23, P<0.01) after inhibition of adenylyl cyclases or removal of extracellular Ca^{2+}, respectively. Rise of $[Ca^{2+}]_{pm}$ evoked by KCl-depolarization caused GFP-Epac2 translocation, but this effect was suppressed by inhibition of adenylyl cyclases. Ca^{2+} nevertheless contributed to cAMP-induced GFP-Epac2 translocation, since the response was reduced by 48±7% (n=43, P<0.01) after removal of extracellular Ca^{2+} and depletion of intracellular Ca^{2+} stores. However, the effect of Ca^{2+} was dual, and high $[Ca^{2+}]_{pm}$ spikes evoked by tetraethylammonium caused temporary dissociation of GFP-Epac2 from the membrane (40±7% reduction, n=32, P<0.01). Epac2 mutants lacking the cAMP-binding or Ras-association domains were unable to translocate and localized constitutively to the plasma membrane and cytoplasm, respectively.
Conclusion: Epac2 localization is dynamically controlled by both cAMP and Ca^{2+} signals. Epac2 recruitment to the plasma membrane should be important for the activation of effectors involved in the regulation of insulin secretion.

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Crosstalk between cAMP- and Zn^{2+}-signalling in insulin secretion
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Background and aims: Glucose stimulation of β-cells evokes synchronized oscillations of the sub-membrane cytoplasmic Ca^{2+} ($[Ca^{2+}]_{pm}$) and cAMP ($[cAMP]_{pm}$) concentrations, which underlie pulsatile insulin secretion (IS). Whereas Ca^{2+} is the principal trigger, cAMP is an important amplifier of IS. Zn^{2+} is present in high concentrations in insulin secretory vesicles and Zn^{2+} co-released with insulin has been proposed to exert a negative feedback on IS by inhibiting Ca^{2+} influx after activation of hyperpolarizing K^+ channels. cAMP turnover is also modulated by Zn^{2+}. In the present study, we investigated how glucose affects the sub-membrane Zn^{2+} concentration ($[Zn^{2+}]_{pm}$) and it's influence on $[Ca^{2+}]_{pm}$, $[cAMP]_{pm}$ and insulin secretion kinetics in single β-cells.
Materials and methods: Simultaneous measurements of $[Ca^{2+}]_{pm}$ and $[Zn^{2+}]_{pm}$ were performed in single MIN6 β-cells co-loaded with the fluorescent indicators Fura Red and FluoZin-3. $[cAMP]_{pm}$ was detected by a fluorescent protein-based translocation biosensor. Another translocation biosensor reported phosphatidylinositol-3,4,5-trisphosphate formation in the plasma membrane ($[PIP_3]_{pm}$) after autocrine insulin receptor activation, and this assay was used as readout for IS from single cells. Changes in $[Zn^{2+}]_{pm}$, $[Ca^{2+}]_{pm}$, $[cAMP]_{pm}$ and $[PIP_3]_{pm}$ were recorded with evanescent wave microscopy.
Results: Glucose stimulation of MIN6 cells induced pronounced $[Ca^{2+}]_{pm}$ and $[cAMP]_{pm}$ oscillations resulting in pulsatile is detected as $[PIP_3]_{pm}$ oscillations. Glucose also elevated $[Zn^{2+}]_{pm}$ and some cells showed synchronized oscillations of $[Zn^{2+}]_{pm}$ and $[Ca^{2+}]_{pm}$, which were particularly prominent after addition of the K^+ channel blocker tetraethylammonium. Blocking of voltage-dependent Ca^{2+} channels with methoxyverapamil or inhibition of Ca^{2+} influx by hyperpolarization with diazoxide prevented both $[Ca^{2+}]_{pm}$ and $[Zn^{2+}]_{pm}$ oscillations. Addition of exogenous Zn^{2+} to glucose-stimulated MIN6 cells resulted in amplification of the $[Zn^{2+}]_{pm}$ oscillations but marked inhibition of the $[cAMP]_{pm}$ and $[PIP_3]_{pm}$ responses as well as suppressed insulin release detected by ELISA. The Zn^{2+}-inhibited $[PIP_3]_{pm}$ oscillations were rescued by 8-Br-cAMP. In the absence of added Zn^{2+} the membrane permeable Zn^{2+} chelator TPEN abolished the Zn^{2+} signal and much amplified the $[Ca^{2+}]_{pm}$, $[cAMP]_{pm}$ and $[PIP_3]_{pm}$ responses to glucose stimulation.
Conclusion: These data reinforce the idea that Zn^{2+} feedback-regulates pulsatile insulin secretion by inhibiting cAMP signaling in β-cells.

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Adenylate cyclase 8 is required for glucose-induced calcium signalling in pancreatic beta cells
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Background and aims: Glucose raises $[Ca^{2+}]_i$ in β-cells, which is a pivotal action of the sugar. Its metabolism leads to an increase in ATP/ADP ratios and the ensuing closure of K_{ATP} channels results in membrane depolarisation and Ca^{2+} influx. Glucose also stimulates cAMP generation, a second messenger considered as an amplifier of the cellular effects of Ca^{2+}. We had previously observed that the Ca^{2+}-activated adenylate cyclase 8 (ADCY8) mediates incretin effect on $[Ca^{2+}]_i$ and that glucotoxicity strongly down-regulates ADCY8 in rat and human islets. In the present study, we addressed the role of cAMP and more specifically of ADCY8 in glucose-induced changes in $[Ca^{2+}]_i$.
Materials and methods: $[Ca^{2+}]_i$ was measured using INDO-1 in clonal INS-1E and primary mouse β-cells. In addition we used, for the first time, a novel electrophysiological approach in β-cells that is the extracellular recording of electrical signals with microelectrode arrays.
Results: In INS-1E cells, glucose-induced increases in $[Ca^{2+}]_i$ were significantly reduced from 330±43 (n=16) to 14±3 nM (n=20; 2p<0.001) by SQ22,536 (100 μM), a general inhibitor of adenylate cyclases. Similarly, the cAMP antagonist Rp-cAMPS (50 μM) reduced glucose effects to 47±12 nM (n=16; 2p<0.001) in INS-1E and from 258±64 to 34±9 nM in primary β-cells (n=21-25; 2p<0.005). In contrast, responses evoked by depolarization with KCl remained unchanged pointing towards a glucose-specific mechanism. The activation of kinases by cAMP, and particularly PKA, seems to be involved as H-89 (40 μM) reduced glucose-evoked $[Ca^{2+}]_i$-increases to 5±2 nM in INS-1E cells (n=11; 2p<0.001). In the same vein, glucose-evoked firing rates recorded with microelectrode arrays were decreased by H-89 in a reversible manner from 1.36±0.18 to 0.10±0.02 Hz in INS-1E (n=33; p<0.001) and from 1.91±0.50 to 0.22±0.15 Hz in mouse β-cells (n=7; p<0.01). Among adenylate cyclases, ADCY8 appeared to play a key role as its overexpression increased the amplitude of glucose-induced increase in $[Ca^{2+}]_i$ from 218±27 to 1005±115 nM and its knockdown reduced the response to 72±10 nM in INS-1E cells (n=7-51; 2p<0.001). In contrast, responses to KCl or thapsigargin remained unaltered. Similarly, knockdown reduced glucose-induced $[Ca^{2+}]_i$ increases in islet cells from 209±36 to 32±5 nM (n=21 each; 2p<0.001) without altering responses to KCl. Finally, preliminary data using $ADCY8^{-/-}$ knockout mice suggest equally a defect in glucose-induced $[Ca^{2+}]_i$ responses and impaired glucose tolerance under normal diet.
Conclusion: Taken together, these results indicate a permissive rather than only amplifying role of cAMP and specifically of ADCY8 in the sequence of events leading from glucose exposure to increases in $[Ca^{2+}]_i$ in clonal and primary β-cells. In line with the down-regulation of this gene during glucotoxicity in rat and human islets, our data underline the pivotal importance of this enzyme in normal β-cell function and potentially in type 2 diabetes.
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Glucose and muscarinic stimulation trigger distinct diacylglycerol signals in pancreatic beta cells
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Background and aims: Diacylglycerol (DAG) is generated in the plasma membrane via phospholipase cleavage of phosphoinositides in response to nutrients and receptor stimuli. Several DAG-activated proteins like protein kinase C, protein kinase D and Munc13 have been implicated in the regulation of insulin secretion. The aim of this study was to characterize the temporal pattern of plasma membrane DAG signaling in insulin-secreting cells exposed to different stimuli.
Materials and methods: A biosensor based on the two adjacent DAG-binding C1 domains of rat protein kinase Cγ tagged to green fluorescent protein (C1aC1b-GFP) was used to monitor DAG in individual MIN6 β-cells. The probe translocates to the plasma membrane upon DAG formation, which was monitored with confocal and evanescent wave microscopy. The cytoplasmic Ca^{2+} concentration in the immediate sub-plasma membrane space was recorded with evanescent wave microscopy in cells loaded with the fluorescent indicator Fura Red.

Results: Confocal imaging of MIN6 β-cells expressing C1aC1b-GFP showed diffuse cytoplasmic fluorescence and a slight accumulation of the probe in the nucleus under basal conditions. Addition of 1 μM of the functional DAG mimetic phorbol myristate acetate caused rapid redistribution of the fluorescence to the plasma membrane. This translocation was detected as a pronounced increase of fluorescence when imaging the plasma membrane with evanescent wave microscopy. Activation of muscarinic receptors with carbachol caused a dose-dependent and sustained, plasma membrane translocation of C1aC1b-GFP with threshold and maximal response at about 0.1 and 100 μM (130±7 % fluorescence increase), respectively, and the half maximal effect at 7±0.7 μM (n=50). Depolarization with 30 mM K^+ caused a rapid increase in the plasma membrane C1aC1b-GFP fluorescence followed by a decline and brief (3-13 s duration), irregular spikes, often originating from a slightly elevated level. This response required influx of Ca^{2+}, since Ca^{2+}-deficient medium containing 2 mM EGTA reversibly removed all spiking. Elevation of the glucose concentration from 3 to 11 mM induced complex changes of the plasma membrane C1aC1b-GFP fluorescence. The majority of cells (55.5%) showed brief irregular high-amplitude (60±12 % fluorescence increase, n=25) spiking like during K^+ depolarization. In 15.5% of the cells, glucose triggered slow (0.31±0.2 min^{-1}), low-amplitude (28±6 % fluorescence increase, n=7) oscillations without spikes, and in 29% of the cells the high-amplitude spikes were grouped into bursts with similar frequency as the slow oscillations. The glucose-induced DAG signaling was Ca^{2+}-dependent and simultaneous measurements revealed that the initial increase of C1aC1b-GFP fluorescence was always preceded by an increase of the sub-plasma membrane Ca^{2+} concentration.
Conclusion: The plasma membrane DAG concentration shows distinct and complex changes in insulin-secreting cells exposed to receptor, nutrient and depolarizing stimuli, probably reflecting different modes of phospholipase C activation. The DAG signaling patterns may differently affect downstream effector proteins involved in the regulation of insulin secretion.

PS 39 Incretins and beta cell mass in rodents

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Preservation of pancreatic beta cell mass in high fat-fed STZ treated mice by the Dipeptidyl peptidase-4 inhibitors Saxagliptin and Sitagliptin
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Background and aims: Saxagliptin is a potent, selective DPP-4 inhibitor, specifically designed for extended inhibition of the DPP-4 enzyme. DPP-4 inactivates incretins that stimulate glucose-dependent insulin secretion. A proposed mechanism of action involves protecting incretins from DPP-4 degradation, thus improving β-cell preservation in conditions of β-cell stress. In this study we investigated the β-cell preservation effects of saxagliptin and sitagliptin at similar exposures in relation to their potencies as DPP-IV inhibitors.
Materials and methods: C57BL/6J mice (12 per group) were placed on a 60% fat diet for 4 weeks prior to 50mg/kg streptozotocin (STZ, ip, daily for 3 days). All animals were randomised based on bodyweight, glucose, insulin and HbA1c. They were dosed with Vehicle (V) Sitagliptin (Sita) or Saxagliptin (Saxa) (10mg/kg/day, po) throughout the study commencing either one week prior to STZ treatment or 1 day after STZ treatment. Glycaemic control was determined by oral glucose tolerance tests (OGTT) 3 weeks post-STZ treatment and by fasting blood glucose 36 days post STZ. Automated imaging and analysis systems were used to determine β-cell mass using terminal formalin-fixed, paraffin-embedded samples taken 36 days after induction of treatment.
Results: Plasma compound concentration measured 24 hours after the final dose gave calculated unbound concentrations 7.9 and 3.1 fold above the Ki value for Saxa and Sita, respectively. Glycaemic control and β-cell mass data are given in the table.

Glycaemic control and beta cell mass following compound treatment

	Vehicle STZ	Sita Pre STZ	Sita Post STZ	Saxa Pre STZ	Saxa Post STZ
FBG (mM)	17.9±1.3	16.1±0.7	15.6±1.2	17.6±1.2	14.4±1.0*
HbA1c (%)	6.8±0.2	6.2±0.2*	6.3±0.2	6.5±0.2	6.1±0.2†
OGTT Glucose $AUC_{Baseline}$ (mM.min)	32.9±1.9	28.4±1.0†	28.4±0.8†	28.3±1.3†	28.3±1.8†
OGTT Insulin $AUC_{Baseline}$ (ng/ml.min)	-0.05±0.14	0.12±0.09	0.09±0.20	-0.05±0.17	0.19±0.17
Beta cell mass (mg)	0.13±0.03	0.16±0.02	0.42±0.06†	0.23±0.03*	0.28±0.05*

(*Data are mean and sem calculated from the residuals of the SAS ROBUSTREG procedure and t-tests undertaken using two-sided tests. *, P<0.05 and †, P<0.01 compared to vehicle*).

In animals treated post STZ, both saxa and Sita significantly reduced glucose $AUC_{Baseline}$ with no effect on insulin $AUC_{Baseline}$ during OGTT. Saxa induced a significant reduction in HbA1c and fasting glucose levels. Both treatments demonstrated a significant improvement in β-cell mass. Mice dosed pre STZ showed that both Saxa and Sita again reduced the glucose $AUC_{Baseline}$. Saxa also demonstrated improvement in β-cell mass compared with vehicle.
Conclusion: Overall both Saxa and Sita showed similar improvements in glycaemic control and β-cell mass preservation in the high fat-fed, STZ mouse model of pancreatic β-cell degeneration. We have demonstrated for the first time that saxagliptin along with improving glycaemic control had a positive impact on β-cell preservation in a rodent model of type 2 diabetes.

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Protective effects of DPP-4 inhibitor against increased beta cell apoptosis with multiorgan glucolipotoxicity by a combination of dietary sugar and fatty acid

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A composition of diet affects metabolic states in diabetes. We investigated diet-induced glucolipotoxicity and effects of des-fluoro-sitagliptin (DFS), a DPP-4 inhibitor, on it, in β cell-specific glucokinase haploinsufficient (*Gck*$^{+/-}$) diabetic mice. We challenged the mice with diet containing a combination of sucrose and oleic acid (SO), or sucrose and linoleic acid (SL) for 25 weeks. In *Gck*$^{+/-}$ mice, but not in the wild-type mice, SL induced impaired insulin secretion in response to glucose. Histochemical analyses revealed that, in *Gck*$^{+/-}$ mice fed SL, β cell mass and proportion of β cells to islet cells were significantly decreased, α cell were dispersed into islet, and both CHOP- and TUNEL-positive β cells were significantly increased, compared to those fed SO. Analysis of mRNA expression showed that CHOP, Bip and SREBP-1c were significantly increased and E-cadherin was significantly decreased in islets of *Gck*$^{+/-}$ mice fed SL, compared to SO. These results indicated that the SL diet induced endoplasmic reticulum (ER) stress and apoptosis in β cells, which resulted in β cell loss and abnormal islet morphology by modification of SREBP-1c and E-cadherin expression. Whereas histology and flow cytometry of epididymal fat indicated increased number of F4/80^{+} CD11c^{+} M1 macrophages and CD8^{+} T cell in SL, compared to SO. We next evaluated DFS monotherapy for increased beta cell apoptosis and glucolipotoxicity in *Gck*$^{+/-}$ mice fed SL diet with 1.1% DFS. Treatment with DFS improved glucose tolerance, protected against β cell apoptosis, restored β cell mass, and normalized islet morphology in *Gck*$^{+/-}$ mice fed SL. DFS normalized the changes of islet mRNA expression of CHOP, Bip, SREBP-1c and E-cadherin by SL diet. DFS also reduced CD8^{+} T cell and M1 macrophage infiltration to epididymal fat. Furthermore, DFS prevented liver steatosis in SL-fed mice. Liver mRNA expression of SREBP-1c and SCD-1 were decreased, and PPARα was increased in DFS treated group. Taken together, DFS protected against metabolic disorders in multiple organs induced by dietary sugar and fatty acid (multiorgan glucolipotoxicity).

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Enhanced proliferation of islet beta cells in spontaneously diabetic GK rats treated with DPP-IV inhibitor (Vildagliptin)

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Background and aims: Progressive decline of beta cell mass is a hallmark of either obese or lean type 2 diabetic patients. Currently, incretins are expected to not only exert insulin secretion but to protect beta cell depletion. It is not entirely clear, however, whether DPP-IV inhibitors have a potential to maintain beta cell survival or to prevent apoptotic processes of beta cells in lean type 2 diabetes. Spontaneously diabetic GK rats are non-obese, typical of lean type 2 diabetes model. In this study, we studied the effects of DPP-IV inhibitor on the islet pathology in GK rats to explore how it can protect the development of beta cell depletion.
Materials and methods: GK rats 4 weeks of age were orally given DPP-IV inhibitor (Vildagliptin 15mg/kg)(VG) twice a day for following 18 weeks. Untreated GK rats were given saline alone. Non-diabetic Wistar rats served controls. At end, all the animals were sacrificed after glucose tolerance test (GTT) (2g/kg) and insulin tolerance test (ITT) (0.5U/kg). Then, pancreases were subjected to evaluation of islet pathology and morphometric analysis of beta cells.
Results: VG treatment significantly suppressed postprandial hyperglycemia and food intake in GK rats, but did not affect blood glucose levels in normal Wistar rats. Gluose intolerance on GTT was also significantly improved in VG-treated GK rats. Lowered insulin secretion in GK rats after meals was significantly improved by VG treatment while no effects on Wistar rats. Insulin resistance detected in GK rats on ITT was significantly improved at 30 min point in VG-treated GK rats, while there was no effect in Wistar rats. The islets in GK rats were atrophic and irregular mixed with macrophage infiltration. VG-treated GK rats exhibited marked hyperplastic islets and less inflammatory changes. Islet morphometry disclosed significant decline of islet volume (48% of Wistar) and beta cell mass (41% of Wistar) in GK rats.VG-treated GK rats recovered the islet volume (120% of Wistar) and beta cell mass (103% of Wistar). Volume density of alpha cells was also decreased in GK rats and corrected by VG-treatment. Proliferation rate of beta cells as revealed by MIB-1 index was significantly reduced in GK rats (21% of Wistar) and VG-treated GK rats recovered the index (5.6 times of untreated GK rats). Apoptotic cells in the islets were not detected in any group, either diabetic or non-diabetic groups. .
Conclusion: Our present study demonstrated that significant recovery of islet size and beta cell volume density was obtained in lean type 2 diabetic GK rats when treated with VG. The effects were mainly caused by enhanced proliferation of beta cells and associated with improved glucose intolerance and insulin secretion.
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GLP-1 counteracts fatty acid inducing expression of PANDER, an apoptotic cytokine secreted from pancreatic islets, through activating Akt pathway

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Background and aims: PANDER is a novel discovered cytokine that is secreted from pancreatic islet cells. It has been demonstrated that overexpression of PANDER induces pancreatic beta-cell apoptosis and dysfunction. To a better understanding of the pathophysiological role of PANDER in the islet beta-cells, we analyzed the effects of fatty acid and the counteracting effects of GLP-1 on PANDER expression and the possible mechanism.
Materials and methods: Beta-TC3 cells were cultured with or without Palmitic acid (PA), and/or GLP-1, Akt inhibitor (Akti). Cell viability was measured with MTT. Annexin-V-FITC/PI FACS was used to analyze cell apoptosis. PANDER mRNA and protein expression were measured by real-time fluorescence quantitative PCR and western blot, respectively.
Results: PA decreases cell viability of pancreatic beta-TC3 cell in a dose dependent manner as measured with MTT, Cell viability reduced one third when cells were treated with 0.8mmol/L PA, and a further decrease of 40% was observed when 1.0 mmol/L PA was used. Cell viability were increased significantly in PA + GLP-1 treated cells comparing to the PA treated cells ($P<0.05$). There were no significant differences between control group and PA + GLP-1 group($P>0.05$). Annexin-V-FITC/PI FACS analysis has showed that the apoptotic rate of beta-TC3 cells were (18.20 ± 2.14)%, (52.73 ± 3.29)% , and (34.49 ± 1.57)% in control, cells treated with PA, and cells treated with PA + GLP-1, respectively. PA increased cell apoptosis significantly (vs control, $P <0. 05$), and GLP-1 rescued cells from PA inducing apoptosis. Comparing to the PA treated cells, the apoptotic cells was significantly lower in cells treated with PA + GLP-1 ($P <0. 05$). PANDER mRNA expression is presented by F value of real time-PCR. The lower of F value, the higher the mRNA expression level. The F values were (1.00 ± 0.00) %, (0.16 ± 0.16) %, and (2.01 ± 0.46) % in control cells, cells treated with PA, and cells treated with PA + GLP-1 respectively. PA increased PANDER mRNA expression significantly (vs control, $P <0. 05$), while GLP-1 decreased PA inducing PANDER mRNA expression (vs PA, $P <0. 05$). Western blot has shown that PA induced 1.5 fold increase of PANDER protein expression, and there is no significant difference of PANDER protein expression between the control and PA+GLP-1 treated cells. GLP-1 reduced PA inducing PANDER protein expression significant (vs PA, $P <0. 05$). The p-Akt protein expression was increased in both of the GLP-1 and GLP-1+PA groups. When Akti was added, there are no significant differences of PANDER protein expression between PA and PA+GLP-1 treated cells were observed. The effect of GLP-1 on PA inducing PANDER expression was reduced.
Conclusion: GLP-1 counteracts PA inducing PANDER expression and rescues beta-cells from PA inducing apoptosis. This effect of GLP-1 on PA inducing PANDER expression is partially through activating the Akt pathway.
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Exendin-4 inhibits palmitate-induced apoptosis in pancreatic beta cells

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Background and aims: Type 2 diabetes is characterized by a progressive decline in the number of insulin-producing beta-cells, largely due to increased cellular apoptosis. Free fatty acids (FFA) are essential energy metabolites in the normal state, but induce beta-cell dysfunction and death when their levels are chronically increased, thus contributing to the pathogenesis of type 2 diabetes. GLP-1 and its long-acting receptor agonist exendin-4 (ex-4) increase the survival of beta-cells exposed to various pro-apoptotic stimuli, including FFA. The aim of this study was to investigate the mechanisms of FFA-induced beta-cell apoptosis and the potential protective effects of GLP-1 mimetics on this response in the rat insulin-secreting cell line INS-1 and in isolated human islets.
Materials and methods: INS-1 beta-cells and isolated human islets were exposed to 0.5 mM palmitate for several h. The effects of ex-4 were evaluated by pre-incubating INS-1 cells with 10 nM ex-4 for 16 h. Protein content and phosphorylation of intracellular signaling intermediates were evaluated by immunoprecipitation and immunoblotting techniques. Gene expression was evaluated by qRT-PCR. Beta-cell apoptosis was quantified by an ELISA assay evaluating oligosome release into the cytosol.
Results: Exposure of both INS-1 cells and isolated human islets to 0.5 mM palmitate, a saturated fatty acid, up to 48 h induced a 2.5-fold increase in cell apoptosis, measured by evaluation of cytosolic oligosomes ($p<0.05$) and cleaved caspase-3 ($p<0.05$). Palmitate induced a 3.5-fold increase in the phosphorylation of JNK1/2, a class of mitogen-activated protein kinases largely involved in beta-cell apoptosis, evaluated by both immunoblotting and immunofluorescence ($p<0.05$), and a 3.8-fold increase in the mRNA levels of the JNK substrate c-jun ($p<0.05$), evaluated by qRT-PCR. Preincubation with 10 μM SP600125, a specific JNK inhibitor, for 2 h prevented palmitate-induced apoptosis ($p<0.05$ vs. palmitate-treated cells). Treatment with 10 nM ex-4 for 16 h inhibited both palmitate-induced activation of JNK1/2 ($p<0.05$ vs. palmitate-treated cells) and apoptosis ($p<0.05$ vs. palmitate-treated cells) in both rat insulin-secreting cells INS-1 and isolated human islets. Furthermore, ex-4 inhibited palmitate-induced phosphorylation of the upstream stress signaling kinases MKK4 and MKK7, which are implicated in JNK activation ($p<0.05$ vs. palmitate-treated cells), and increased the protein content of Islet-Brain 1 (IB1), a blocker of the stress-induced JNK pathway ($p<0.05$).
Conclusion: Palmitate induces apoptosis of human and rat beta-cells by activating JNK1/2, and the GLP-1 analog ex-4 prevents palmitate-mediated apoptosis, at least in part by interfering with the JNK activation pathway. These results provide evidence that the ability of ex-4 to prevent FFA-induced apoptosis involves inhibition of the JNK pathway, identifying an important mechanism by which GLP-1 receptor agonists may halt beta-cell death triggered by metabolic abnormalities.
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PEGylated PYY_{3-36} has beneficial effects on glucose handling and exhibits islet sparing effects in db / db mice

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Background and aims: NPY2-receptor peptide agonists suppress appetite in animal models of obesity, and therefore show great promise as treatment for obesity. However, their anti-diabetic potential has not been fully explored. In this study we investigated the anti-diabetic potential of long acting PEGylated PYY_{3-36} (PEG-PYY).
Materials and methods: Acute effect of PEG-PYY (3, 10, 20 and 30 mg/kg) on fasting glucose 26 h after a single subcutaneous (sc) administration was measured in diabetic 11-week old female *db/db* mice (Jackson Laboratories, Bar Harbor, ME). An oral glucose tolerance test was conducted to determine the effects on glucose disposal. We next examined the effects of PEG-PYY (sc, q2d) administration on glucose handling and islet morphology following sub-chronic dosing for 6 weeks in 9-week old *db/db* mice. The effects of 3-week PEG-PYY treatment (q2d) on body weight and insulin sensitivity were measured in male C57Bl/6 DIO mice (Jackson laboratories).
Results: PEG-PYY exhibited high NPY2-receptor selectivity (>100-fold) against related NPY-receptors. In leptin receptor deficient diabetic *db/db* mice, a single sc dose of PEG-PYY produced a 61% reduction in fasting blood glucose levels at 26 h post-dose. In addition, there was a significant improvement in glucose handling observed following an oral glucose challenge. Similar benefits were observed following sub-chronic dosing with PEG-PYY. In 9-week old *db/db* mice, PEG-PYY (20 mg/kg) injected every other day for 6-weeks improved glucose handling and had an islet sparing effect. A substantial improvement in islet morphology and insulin immunoreactivity was observed in PEG-PYY treated mice. In addition to its anti-diabetic benefits, PEG-PYY displayed robust anti-obesity effects as expected. Following sub-chronic dosing in DIO mice, a sustained reduction in body weight gain (12% @ 20 mg/kg) and an improvement in insulin sensitivity were observed. Significant improvements in levels of glucose, insulin, triglycerides and cholesterol were also noted.
Conclusion: In summary, we have observed that PEG-PYY improves glucose handling following an acute dose diabetic *db/db* mice. Sub-chronic treatment with PEG-PYY delayed the onset of diabetes in mildly hyperglycemic *db/db* mice and maintained islet health. These benefits of PEG-PYY likely involve its effects on insulin sensitivity, food intake, weight gain and lipid metabolism. Thus long acting PYY analogs have the potential to be developed as treatments for diabesity, with the added benefit of improved lipid parameters.

PS 40 Hypoglycaemia in type 2 diabetes

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A basal–bolus regimen of insulin glargine and insulin glulisine results in a lower rate of hypoglycaemia relative to endpoint HbA_{1c} versus twice-daily premixed insulin in type 2 diabetes patients

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Background and aims: We recently demonstrated, in the GINGER study, that an intensified basal–bolus regimen using insulin glargine (GLAR) and insulin glulisine (GLU) provides significantly superior glycaemic control compared with premixed insulin therapy (ΔHbA_{1c} reduction 0.5%; Table) in a population with long-standing insulin-treated type 2 diabetes mellitus (T2DM). Although a difference in rates of hypoglycaemia favouring the basal–bolus arm was present, this was not statistically significant. It is well known that greater improvements in HbA_{1c} are associated with higher rates of hypoglycaemia. The aim of this analysis was to investigate the rate of hypoglycaemia relative to the achieved glycaemic control in terms of HbA_{1c}.
Materials and methods: In this 52-week, open, randomized, multinational, multicentre clinical trial, mealtime GLU and once-daily GLAR (n=153) was compared with an optimized conventional therapy of twice-daily premixed insulin (n=157) in T2DM patients inadequately controlled with their previous premixed insulins. In the premixed insulin group, 63 subjects received a combination of NPH/aspart. At baseline, the characteristics of the total study population (49% female) were (mean ± standard deviation): age, 61 ± 8 years; body mass index, 30.1 ± 3.7 kg/m^2; diabetes duration, 13 ± 6 years; and insulin use, 5 ± 4 years. We used negative binomial regression analysis to model hypoglycaemia outcomes by endpoint HbA_{1c}, adjusting for baseline HbA_{1c} and diabetes duration, taking zero inflation into account.
Results: The subjects with GLAR/GLU therapy had a 24.5% lower rate of overall hypoglycaemia (1399.17 ± 2324.24 vs 1854.31 ± 3694.83 events per patient year for GLAR/GLU and premix, respectively; unadjusted rate; Table) than the whole premix group (p=0.1211 from the adjusted model) and a 43.3% (p=0.0196) lower rate of overall hypoglycaemia than the NPH/aspart subgroup. When the analysis only included patients with at least one episode of hypoglycaemia during the study, the subjects with GLAR/GLU therapy had a 26.5% (p=0.0442) and 40.7% (p=0.0086) lower rate of overall hypoglycaemia than the whole premix group and NPH/aspart subgroup, respectively. Analyses with confirmed hypoglycaemia yielded similar results.
Conclusions: In the GINGER study, basal–bolus treatment with GLAR/GLU displayed a lower rate of hypoglycaemia in relation to endpoint HbA_{1c} compared with premixed insulin therapy. This was more pronounced in the analysis comparing the NPH/aspart-treated subgroup with basal–bolus treatment. Therefore, a basal–bolus regimen with insulin analogues is safer than premixed insulin therapy in T2DM patients with long-standing diabetes, when considering endpoint HbA_{1c}.

Table: Hypoglycaemia and HbA1c

	Insulin glargine/insulin glulisine	Premix	
		Al	NPH/aspart
Hypoglycaemia (events per 100 patient–years; safety population)			
	n=153	n=157	n=63
Overall hypoglycaemia	1399.17 ± 2424.24	1854.31 ± 3694.83	2468.39 ± 4853.41
Confirmed hypoglycaemia*	1129.52 ± 2286.84	1548.67 ± 3630.64	2172.21 ± 4868.58
HbA_{1c} (mean ± standard deviation; full analysis set)			
	n=153	n=157	n=61
Baseline HbA_{1c}, %	8.62 ± 0.83†	8.51 ± 0.86	8.44 ± 0.90
Endpoint HbA_{1c}, %	7.31 ± 1.16	7.71 ± 1.14	7.74 ± 1.31
Baseline to endpoint change, %	−1.31 ± 1.19‡§	−0.80 ± 1.01	−0.70 ± 1.08
Adjusted mean difference in HbA_{1c} change versus GLAR/GLU, % (95% confidence interval)	–	−0.476 (−0.714, −0.238)	−0.589 (−0.933, −0.246)

*Confirmed hypoglycaemia was defined as an event with confirmed blood glucose level ≤60 mg/dL; †p=0.3315 compared with premix; ‡p=0.0001 compared with premix; §p=0.0009 compared with NPH/aspart

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Detection of patient-specific intra-day patterns among hypoglycaemia episodes using continuous glucose monitoring

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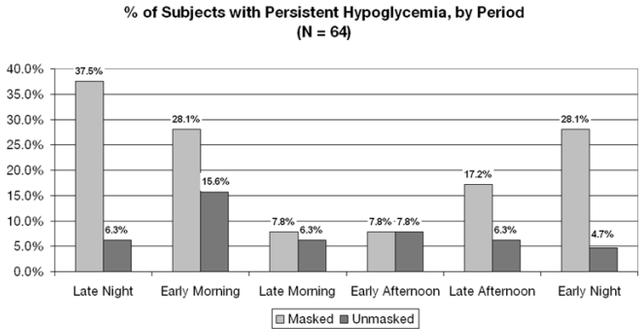
Background and aims: Incidence of hypoglycemia can be affected by insulin dosing, exercise, meals and sleep. Patterns in lifestyle and treatment decisions can result in consistent hypoglycemic patterns. Rapid computational screening of patient data can identify times of day with significant temporal patterns, which can then inform self-management adjustments that lead to improved glycemic control. A computational method for identifying patient-specific patterns in hypoglycemic episodes is proposed and applied to CGM data from a 40-day Freestyle Navigator® System home-use study to evaluate the impact of unmasked CGM readings on hypoglycemic patterns.
Materials and methods: 64 (T1DM=46, T2DM=18, all insulin users) subjects were selected for analysis based on high data availability and presence of at least 2 hours of hypoglycemia (<3.89 mmol/L) over the 40 day study. Subjects had glucose data masked for the first 20 days and unmasked for the following 20 days. During the masked phase, subjects were not able to see their CGM glucose values or trends and did not have glucose threshold or projected alarms available. CGM data from each subject's 20-day phases are divided into 2,880 ten-minute segments and categorized as hypoglycemic or not. For each subject, the number of ten-minute hypoglycemic segments is counted in each of six periods of the day (Early Morning: 4:00-8:00, Late Morning: 8:00-12:00, Early Afternoon: 12:00-16:00, Late Afternoon: 16:00-20:00, Early Night: 20:00- 0:00, Late Night: 0:00-4:00). Any period of the day with 30 or more hypoglycemic segments (corresponding to an average of 1.5 segments, or 15 minutes of hypoglycemia, in a given four hour period per day) is labeled as a persistent hypoglycemia pattern for that subject. The proportions of subjects with such patterns during the masked and unmasked phases were compared for each period of the day using pooled-proportion *z*-tests.
Results: The proportion of subjects with persistent hypoglycemia patterns differed significantly between the masked and unmasked phases in the Early Night (28%, 5%, p=0.0003) and Late Night (38%, 6%, p < 0.0001) periods. In the Early Morning (28%, 16%, p=0.0872), Late Morning (8%, 6%, p=0.7296), Early Afternoon (8%, 8%, p=1.0000) and Late Afternoon (17%, 6%, p=0.0544) periods, the proportion of subjects with persistent hypoglycemia patterns did not differ significantly.
Conclusion: The proposed computational method is useful for identifying periods of the day with hypoglycemic patterns on a per-subject basis. When used to compare masked and unmasked phases of a CGM home-use study, the incidence of persistent hypoglycemia was found to be significantly lower during the Early Night and Late Night periods of the unmasked phase relative to the masked phase. The method can be extended to identify other glycemic episodes of interest (such as hyperglycemia) over different time periods (e.g. days of the week). Prospective studies would be needed to evaluate the method's ability to improve therapy decisions.



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Relationship of hypoglycaemia with medication, discontinuation and costs in type 2 diabetes mellitus patients

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Background and aims: Hypoglycemia is a common and potentially severe adverse effect of antidiabetic treatment and poses significant barrier to main-

taining tight blood glucose control. (Amiel, 2008; Cryer, 2009) This study investigates the clinical and economic effect of hypoglycemia in type 2 diabetes mellitus (T2DM) patients initiated on oral and/or insulin antidiabetic drugs (OADs). Risks of hypoglycemia, likelihood of discontinuation, medical costs and medication type are compared between patients diagnosed with hypoglycemia vs. no hypoglycemia.
Materials and methods: T2DM patients initiated on OADs were identified in the Ingenix IMPACT Database (1999-2008). Included patients were ≥18 years old and had ≥1 year of continuous eligibility following the index date (first OAD prescription). Hypoglycemia risk factors in the subsequent 6-month interval were examined with multivariate Cox PH models adjusting for demographic characteristics and time-varying covariates (diabetes treatments and co-morbidities). The effect of hypoglycemia and other factors on antidiabetic treatment discontinuation was analyzed using GEE models with repeated measures. Annual cost outcomes for hypoglycemia were analyzed using GLM regressions.
Results: A total of 212,061 T2DM patients with ≥1 OAD treatment were identified, with 4,860 (2.3%) patients having a hypoglycemia diagnosis in the first year following the index date. Use of sulfonylureas (HR=1.58, p<.0001), insulins (HR=1.77, p<.0001), meglitinides or alpha-glucosidases (HR=1.27, p<.0001) was associated with a significant increase in hypoglycemia risk in the next 6-month interval. Use of DPP-4 inhibitors (HR=0.79, p=.0141), TZDs (HR=1.06, p=.0133) or metformin (HR=0.99, p=.8203) had minimal effect on hypoglycemia risk. Hypoglycemia diagnosis in a given 6-month interval significantly increased the likelihood of treatment discontinuation (OR=1.27, 95% CI=1.23, 1.32) within the same and the next 6-month interval (OR=1.14, 95% CI=1.09, 1.19). Moreover, for patients with a hypoglycemia diagnosis, average annual total costs (medical+drug costs, $18,273 vs. $8,908, p<.0001) and diabetes-related costs ($8,969 vs. $3,220, p<.0001) were significantly higher than for those without a diagnosis.
Conclusions: Patients experiencing hypoglycemia are at a higher risk of therapy discontinuation while hypoglycemia risk varies among treatments; insulin and SUs are associated with the most risk within the short term. The cost impact is twice as high with hypoglycemia compared to no hypoglycemia due to significantly higher total medical and diabetes-related costs.
Supported by: TPNA, Inc.

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Impact of HbA_{1c} and sulfonylurea use on hypoglycaemia in type 2 diabetes mellitus

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Background and aims: This study examined the relationship between HbA1c level, oral antidiabetics, and hypoglycemia among type 2 diabetes mellitus (T2DM) patients receiving sulfonylureas (SU).
Materials and methods: A retrospective claims analysis of the I3 Innovus population from Jan 1, 2002-Dec 31, 2008 was conducted on T2DM patients with no history of diabetes-related complications or recent (3 months prior) T2DM medication changes (N=10,291). Patients receiving SU at baseline were included and classified as high or low dose based on the median SU dose (10 mg glyburide or glipizide, 4 mg glimepiride). Dose was classified as high dose if the average daily dose was above the median dose. In addition to SU, insulin use was also assessed. The outcome measure was incidence of diabetes-related hypoglycemic events, derived from claims with an ICD-9 code for hypoglycemia. Cox proportional hazards models measured the association between HbA1c and hypoglycemia, adjusting for clinical and demographic characteristics.
Results: At baseline for all 10,291 SU patients (mean age 57; 57% male; mean HbA1c 7.8%), 35% (n=3,628) of patients received high-dose SU; 7.7% (n=788) received SU and insulin. Over the average 3 year follow-up period, 7.2% (n=736) had a hypoglycemic event. The likelihood of a hypoglycemic event increased monotonically by HbA1c. Specifically, those with high baseline index HbA1c (≥9.0) were 47.2% more likely to have a hypoglycemic event in the follow-up period (OR 1.472, CI=1.094, 1.982). Similarly, patients receiving high-dose SU were 35.7% more likely to have hypoglycemic events than low-dose patients, and those patients on insulin in addition to SU had an increase in likelihood of 72.1% (Table 1).
Conclusion: The results indicate that high-dose SU and increased HbA1c are associated with increased likelihood of hypoglycemic events.

Table 1. Predictors of Hypoglycemia in Patients Receiving SU

Variables	OR	95% Wald Confidence Limits	
HbA1c Level (6.5 - 6.9% as Ref)			
<6.5%	1.238	0.924	1.659
7.0 - 7.9%	1.234	0.926	1.645
8.0 - 8.9%	1.242	0.913	1.689
≥ 9%	1.472*	1.094	1.982
High-Dose SU	1.357**	1.152	1.598
Age (yrs)	1.032**	1.025	1.040
Male Gender	0.906	0.777	1.056
Concomitant Diabetes Medications			
Insulin (+ Any SU)	1.721**	1.351	2.192
Other (+ Any)	1.116	0.931	1.338
Charlson Index**	1.199**	1.119	1.284

* p <0.05 ** p <0.01

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Risk factors for symptomatic hypoglycaemia in people with type 2 diabetes mellitus initiated on insulin therapy: evidence from the CREDIT study

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Background and aims: Hypoglycaemia has been suggested to be a potential causative factor in the development of diabetes-associated cardiovascular disease (CVD). We evaluated risk factors for symptomatic hypoglycaemia in the CREDIT study, a 314-centre, multinational, non-interventional, prospective study designed to investigate the effects of insulin initiation and long-term glycaemic control on CVD risk, with 3031 type 2 diabetes mellitus (T2DM) patients enrolled.
Materials and methods: Patients with at least one documented episode of symptomatic hypoglycaemia between 6 months and 1 year after initiating insulin were identified. Potential factors associated with hypoglycaemia (including patient demographics, medical history, CVD risk factors, glycaemic control and diabetes treatment) were described and assessed in a multivariable stepwise logistic regression. To enter and retain each factor in the model a p value of 0.20 and 0.05, respectively, was required. Multivariable analysis was adjusted for country.
Results: Of 2510 patients included in this analysis, 504 (20.1%) experienced at least one documented episode of symptomatic hypoglycaemia over the analysis period. In the final multivariable model, body mass index (BMI) at insulin initiation and HbA_{1c} level following 1 year of treatment were inversely correlated with symptomatic hypoglycaemia (Table). Each 1% increment in HbA_{1c} levels at 1 year was associated with a 25% reduction in the risk of symptomatic hypoglycaemia over the course of the study. Each 1 kg/m^2 increment in BMI at initiation was associated with a 3% reduction in symptomatic hypoglycaemia. Risk of hypoglycaemia was significantly lower with basal insulin alone than with other insulin strategies including premixed insulin and short-acting insulin alone. Non-intensive versus intensive insulin therapy and physical inactivity versus activity were also associated with a lower rise of symptomatic hypoglycaemia.
Conclusion: In patients with T2DM recently initiated on insulin therapy, low BMI at insulin initiation, low HbA_{1c} and insulin regimen at 1 year, physical activity and use of intensive insulin therapy were significant risk factors for symptomatic hypoglycaemia. The use of basal insulin alone was associated with a lower risk of symptomatic hypoglycaemia than other insulin regimens.

Table: Multivariable model of risk factors for documented symptomatic hypoglycaemia

Variable	Odds ratio*	95% CI	p value
BMI (kg/m^2) at insulin initiation	0.968	[0.948; 0.988]	0.0021
Physical activity: no vs yes	0.735	[0.589; 0.917]	0.0063
Normalized HbA_{1c} (%) at 1 year	0.752	[0.687; 0.822]	<0.0001
Insulin used at 1 year			
Basal + short-acting vs basal alone	1.972	[1.412; 2.755]	<0.0001
Premixed insulin vs basal alone	1.730	[1.298; 2.307]	0.0002
Short-acting alone vs basal alone	1.766	[1.113; 2.802]	0.0158
Other insulin† vs basal alone	2.381	[1.336; 4.243]	0.0033
Intensified insulin therapy: yes vs no	1.424	[1.109; 1.830]	0.0057

*Odds ratios were adjusted for between-country differences; †Any insulin regimen not covered by the four other insulin categories; BMI=body mass index; CI=confidence interval

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The average length of hospital stay for a hypoglycaemic event in patients diagnosed with type 1 diabetes mellitus and type 2 diabetes mellitus

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Background and aims: Hypoglycemia, abnormally low blood glucose levels, can occur in patients diagnosed with both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), and is often associated with intensive treatment strategies. The resulting outcomes range from hunger, dizziness and confusion, to seizure, unconsciousness and rarely, brain injury. Patients who experience a severe hypoglycemic event are commonly hospitalized and therefore contribute to the health service burden. The aim of this study was to determine and examine the comparative length of hospital stay associated with hypoglycemic events in patients with T1DM and T2DM.
Material and methods: Routine hospital data were obtained from the Hospital Episode Statistics (HES) data warehouse, which contains anonymized, continuously collected data from all English hospitals. All patients with a primary diagnosis of hypoglycemia (ICD10 E162) between 1999 and 2006 were selected. Only a patient's first hypoglycemic event was included in the study, subsequent events were removed. Patients were subsequently categorized according to their type of diabetes using previous hospital admission definitions within the time period; T1DM (ICD10 E10) or T2DM (ICD10 E11). Patients whose history included a diagnosis of both T1DM and T2DM were removed from the study. The single outcome measure was length of hospital stay in days.
Results: The number of patients experiencing a hypoglycemic event within the dataset was 15,995 for T1DM and 21,736 for T2DM. Patients with T2DM were associated with significantly (p<0.0001) longer mean length of hospital stay for hypoglycemic their events (6.0 days; SD 8.9), compared to those with T1DM (3.2 days; SD 11.1).
Conclusion: The mean length of hospital stay associated with hypoglycemic events was 88% greater for those patients diagnosed with T2DM when compared to patients diagnosed with T1DM. Use of therapies for T2DM that are associated with a reduced risk of hypoglycemia can reduce the number of hypoglycemic events in these patients, and consequentially reduce length of hospital stay, and ultimately the burden to the health service.

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Severe sulphonylurea-induced hypoglycaemia - a problem of uncritical prescription and deficiencies of diabetes care in geriatric patients

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Background and aims: Severe sulphonylurea-induced hypoglycaemia (SH) remains a life-threatening and underreported condition. We investigated the incidence of SH and clinical characteristics of patients with type 2 diabetes mellitus (T2DM) to demonstrate typical risk constellations.
Materials and methods: In a population based observational study, all consecutive cases of SH in the period 2000-2009 in a German area with 200.000 inhabitants were registered. Severe hypoglycaemia was defined as a symptomatic event requiring treatment with intravenous glucose and was confirmed by a blood glucose measurement of <50 mg/dl.
Results: A total of 1.419 cases of severe hypoglycaemia, 141 (10%) of them having been treated with sulphonylureas, were registered among the 103.256 patients who attended the medical emergency department. A mean incidence of 7 episodes of SH per year and 100.000 inhabitants was registered. The 139 hypoglycaemic individuals with T2DM (mean plasma glucose level of 31±10 mg/dl) had been treated with glimepiride (n=98), glibenclamide (n=40) or gliquidone (n=1). Neither preparation showed a constant dose-effect relationship, SH occurring within a wide dose range. The patients were characterised as follows: age 77.5±9.4 years, duration of diabetes 11±7 years, BMI 26.3±4.9 kg/m2, HbA1c 6.6±1.3%, creatinine clearance 46±24 ml/min. with renal insufficiency in 73%, comedication 7±3 drugs. 27% of patients with SH received additional drugs which were also main substrates of CYP2C9 the genetically polymorphic cytochrome P450 enzyme being responsible for the hepatic metabolism of SUs. In particular, they received torasemide, clopidogrel, phenprocoumon, diclofenac, phenytoine or fluvastatine. Two third of all subjects lived independently at home whereas one third was cared for by a home nursing service or received care in nursing homes. 30% had participated in diabetes education programs. In 31% systematic blood glucose monitoring was performed.
Conclusion: The tight glycaemic control (HbA1c 6.6%) in our patients can be regarded as highly predictive for recurrent antecedent hypoglycaemic episodes that were not perceived either by the patients themselves or by their relatives or care staff. Uncritical prescription of sulphonylureas neglecting crucial contraindications - particularly renal insufficiency - and deficiencies of diabetes care contributed substantially to the risk of SH in the mainly geriatric patients. There is a need for alternative therapeutic concepts that minimize the risk of hypoglycaemia in geriatric patients with T2DM.

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The PANORAMA pan-European Survey: impact of severe and non-severe hypoglycaemia on quality of life and other patient reported outcomes in patients with type 2 diabetes

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Background and aims: Hypoglycaemia is a side effect of glucose-lowering treatment that can cause distress and injury, and may damage quality of life (QoL) in patients with type 2 diabetes (T2D). PANORAMA is a large pan-European cross-sectional survey (NCT00916513) aimed at assessing patient reported outcomes in patients with T2D treated with glucose-lowering therapies. The present analysis compares data on QoL, treatment satisfaction and fear of hypoglycaemia in patients with T2D in relation to their experience of hypoglycaemia.
Materials and methods: Patients with T2D were randomly or consecutively selected from physician practices (mainly in primary care) in 8 countries (Belgium, Germany, Greece, Italy, the Netherlands, Spain, Turkey, and the UK). Eligible patients were ≥40 years of age, with a diagnosis of T2D for >1 year prior to study entry and an available medical record at the clinic of >1 year. All patients received dietary and exercise advice. Most patients were also being treated with either oral hypoglycaemic agents (OHAs) or injectables (insulin and GLP-1 receptor analogues) with or without OHAs. Treatment type was unchanged in the previous 3 months. Patients completed the Audit of Diabetes-Dependent Quality of Life (ADDQoL), Diabetes Treatment Satisfaction Questionnaire (DTSQ) and the worry subscale of the Hypoglycaemic Fear Survey-II (HFS-II). Data on history of severe and non-severe hypoglycaemic episodes were collected.
Results: 5,156 patients were included in the study between June and November 2009: 47.8% women; mean age 65.9 years (SD 10.3). Mean time since diagnosis was 9.0 years (SD 7.4). Patients who had experienced at least one episode of severe hypoglycaemia in the past 12 months showed significantly more negative impact of diabetes on QoL (ADDQoL, p<0.001), less satisfaction with treatment (DTSQ, p=0.004) and greater fear of hypoglycaemia (HFS-II, p<0.001) than patients who had not experienced severe hypoglycaemia. Similar differences were found on all three measures for a comparison of patients who experienced more than one episode of non-severe hypogly-

caemia per month with those who experienced one or fewer episodes per month (all p<0.001).
Conclusion: The occurrence of both severe and non-severe hypoglycaemia in T2D is associated with greater negative impact of diabetes on QoL, less treatment satisfaction, and greater fear of hypoglycaemia, and may be an important barrier to optimal glycaemic control in some patients.

Mean (SD) ADDQoL average weighted impact score, DTSQ treatment satisfaction score and HFS-II fear of hypoglycaemia score for patients who experienced ≥1 vs 0 episodes of severe hypoglycaemia, and patients who experienced >1 vs ≤1 episodes of non-severe hypoglycaemia in the last year

Episodes of hypoglycaemia in past 12 months	n	Mean (SD) score		
		ADDQoL[$]	DTSQ^	HFS-II[§]
≥1 severe	313	-1.0 (0.6)	26.3 (7.0)	30.4 (18.1)
0 severe	4,831	-0.6 (0.5)	30.1 (6.0)	12.9 (15.3)
Difference[1]		-0.22**	-1.14*	10.26**
>1 non-severe/month	889	-0.8 (0.6)	28.2 (6.8)	21.9 (17.7)
≤1 non-severe/month	4,258	-0.6 (0.5)	30.2 (5.9)	12.2 (15.1)
Difference[2]		-0.18**	-1.07**	7.34**

[$]average weighted impact score: range -9 (most negative impact) to +3 (most positive impact of diabetes on QoL); ^range 36 (very satisfied) to 0 (very dissatisfied); [§]range 72 (most fearful) to 0 (least fearful)
[1]Patients with ≥1 episode of severe hypoglycaemia - patients with 0 episodes of severe hypoglycaemia.
[2]Patients with >1 non-severe hypoglycaemic episode/month - patients with ≤1 non-severe hypoglycaemic episode/month.
Differences were calculated with mixed-effects regression models adjusting for centre.
*p=0.004; **p<0.001
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Effect of erythropoietin on cognitive performance, symptoms and counter-regulation during hypoglycaemia in patients with type 1 diabetes
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Background and aims: The incidence of severe hypoglycaemia in type 1 diabetes has not decreased over the past decades. New treatment modalities to minimise episodes of hypoglycaemia and attenuate hypoglycaemic cognitive dysfunction are needed. We studied if treatment with the potentially neuroprotective hormone erythropoietin (EPO) enhances cognitive function during hypoglycaemia.
Materials and methods: Eleven type 1 diabetic subjects with hypoglycaemia unawareness and at least two episodes of severe hypoglycaemia in the last year underwent a double-blind, randomised, balanced, cross-over study evaluating the effect of 40.000 IU of EPO on cognitive function, hypoglycaemic symptoms and counter-regulatory response during clamped hypoglycaemia. EPO or placebo were injected intravenously six days before the two experiments which were separated by at least six weeks. Two reaction time tests (CalCAP), Stroop's Colour and Word Test and a Trail Making Test (TMT) were used to evaluate cognitive function. The effects of EPO on cognitive function, hypoglycaemic symptoms and counter-regulatory hormones were assessed by analysis of covariance with baseline values and blood glucose during hypoglycaemia as co-variates.
Results: Mean (SD) plasma glucose concentration during hypoglycaemia was 2.2 (0.3) mmol/l on the EPO day and 2.0 (0.3) mmol/l on the placebo day (p=0.17). Compared with placebo, EPO treatment was associated with (parameter estimate (95% C.I.): a) reduction in the two reaction times during hypoglycaemia: 5 msec (-79 - 68; p=0.88) and 44 msec (-91 - 4; p=0.07); b) reduction in errors in the two reaction time tests: 0.3 (-1.4 - 2.1; p=0.68) and 4.1 (-7.4 - -0.9; p=0.017); c) increase in completed items in Stroop's Word, Colour and Word-Colour Test: 2 (-6 - 11; p=0.58), 1 (-6 - 8; p=0.71), and 2 (-4 - 7; p=0.51), respectively; d) reduction in completion time in TMT: 3 sec (-45 - 40; p=0.88); and e) reduced neuroglycopenic and autonomic symptom scores: -0.9 points (-3.8 - 2.0 points; p=0.46) and -0.6 points (-5.5 - 4.3; p=0.8), respectively. Peak values of adrenaline, cortisol, growth hormone and glucagon did not differ between EPO and placebo days.
Conclusion: In patients with type 1 diabetes and hypoglycaemia unawareness, treatment with EPO is associated with a beneficial effect on cognitive function in only one of six tests of cognitive function. Hypoglycaemic and counter-regulatory hormonal responses were not changed by EPO treatment. However, it cannot be ruled out that alternative timing and dosage of EPO or new non-erythropoietic EPO analogues may be more efficient in enhancing cognitive function during hypoglycaemia.
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The effect of diabetes and its control on susceptibility to learned helplessness in streptozotocin-induced diabetes rats
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Aims: In order to examine the mechanism linking diabetes to depression, specifically whether diabetic rats in good glycemic control versus those in hyperglycemic, or subject to exposed alternative periods of hyper and hypoglycemia, are different at risk of affective disorder. We induced learned helplessness, as model of depression, is indicated the rate of cognitive processing.
Methods: Using the streptzotocin rats receiving insulin NPH or saline, totally 37 rats, were divided into 4 types of glycemic control groups, Group A (good), B (hypo-hyper) ,C (untreated), D (controls). The bodyweight, the blood glucose concentration and HbA1c were measured. And all animals were placed in a learned helplessness paradigm, Forced swimming test (FST): the scorer would rate the rat's behavior each 5 second period, as one of the following four behaviors: immobility and another action. We examined the differences in the length of immobility (a key marker of learned helplessness) with the analysis using Tukey-Kramer multiple comparison.

Results: Glycemic control: Group A had nearly kept smaller change of blood glucose throughout the day. Group B of blood glucose showed a very steep fall from 475.8 ± 193.2 (mean ± SD) mg/dl to 42.8 ± 22.6 mg/dl within three hours after the insulin injection. Mean HbA1c of the diabetic group without insulin treatment significantly increased from 5.9 ± 1.3 to 8.7 ± 4.0 % ($p < 0.0001$). In diabetic groups receiving insulin injection once a day or twice a day, the level of HbA1c significantly decreased from 5.6 ± 1.0 to 3.8 ± 0.6 % and 6.4 ± 1.7 to 3.7 ± 0.5 % respectively. There was no difference in the levels of HbA1c before treatment and after treatment between the groups with one daily injection and two daily injection of insulin. The bodyweight in A and B groups was not significantly different compared with that in non diabetic control group ($P = 0.001, 0.001$). Group C is significantly low compared with that in Group D (< 0.0001). FST: There were trends for counts of immobility to be higher in all 3 diabetic groups compared to Group D, especially in Group B, the mean count of immobility is significantly high compared with Group D (< 0.001). There is no significant difference between 4 groups in the mean counts of swimming. The climbing was significantly low in Group C compared to Group D (< 0.001).
Conclusion: The rats which had been exposed to hypoglycemia and hyperglycemia are more likely to develop learned helplessness than good glycemic control rats. These investigations are thought to be interesting to examine the relationship between diabetes and depression, and suggested that acute varieties in glycemic control could be the mechanism of susceptibility to affective disorder.

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Physiological and performance differences between drivers with type 1 diabetes with and without a history of recurrent hypoglycaemia driving mishaps

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Background and aims: In Europe and the U.S. collisions are more than twice as common among drivers with type 1 diabetes (T1DM) than spouses who do not have diabetes. This increased risk appears to be attributable to a subgroup of drivers with T1DM. The hypothesis tested is that this vulnerable subgroup is more at risk for hypoglycemia and its disruptive effects on driving ability.
Materials and methods: Thirty-eight drivers with Type 1 diabetes, 16 with (+History) and 22 without (-History) a recent history of recurrent hypoglycemia-related driving mishaps, drove a virtual reality driving simulator and watched a videotape of someone else driving a simulator for 30 minute periods. Driving and video testing occurred in a double-blind, randomized, crossover manner during euglycemia (5.5 mmol/L) and progressive hypoglycemia (3.9 down to 2.5 mmol/L). Subjects completed a brief neuropsychological test battery pre and post the euglycemia trial, and during the hypoglycemia trial they completed the battery at euglycemia immediately before and after and at hypoglycemic naidar. Examiners were blind to which subjects were +/-History, while subjects were blind to their blood glucose levels and targets.
Results: Groups did not differ on gender, driving history, HbA1c, insulin dose, BMI, hypoglycemia awareness or frequency of self-treatment during hypoglycemic driving in the laboratory study. +History subjects did report more episodes of severe hypoglycemia in the previous 12 months ($p<.03$). During euglycemia, +History participants reported more autonomic and neuroglycopenic symptoms ($p<.01$), performed worse on neuropsychological tests ($p<.01$) and tended to require more dextrose infusion to maintain euglycemia with the same insulin infusion ($p<.09$). During progressive hypoglycemia, these subjects demonstrated less epinephrine release ($p=.02$), greater driving impairments ($p=.03$), and performed worse on neuropsychological tests ($p<.01$).
Conclusion: Current findings support the speculation that there is a subgroup of type 1 diabetes drivers more vulnerable to experiencing hypoglycemia-related driving mishaps. This increased vulnerability may be due to possibly greater carbohydrate utilization, rendering them more vulnerable to experiencing hypoglycemia. During hypoglycemia they may release less hormonal counter-regulation, leading to more profound hypoglycemia, and more neuroglycopenia, rendering them more vulnerable to hypoglycemia-impaired driving. Such individuals may be counseled to treat pending or actual hypoglycemia more aggressively and early immediately before and during driving.
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PHUn - Psychopathology of Hypoglycaemia Unawareness

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Background and aims: A structured education programme teaching flexible insulin therapy to people with Type 1 diabetes (T1DM) demonstrated that hypoglycaemia awareness could be regained in 48% of hypoglycaemia-unaware patients who had attended a 5 day course. However neuroimaging data show abnormal reward responses in people with T1DM and hypoglycaemia unawareness (HU). And there remain 52% HU patients who do not regain awareness from education. Indeed, this group show less behaviour change with current educational strategies and are at high risk of severe hypoglycaemia. Our study was designed to examine the meanings people with HU assign to their condition. The study aims to: 1. use clinical interviews to identify cognitive biases; 2. construct a theoretical account which explains why the cognitive abnormality does not self-correct; 3. develop specialised psychological interventions to address the cognitive bias; 4. test the efficacy of the interventions in randomised controlled trials and 5. make the treatments more broadly available through dissemination studies. This abstract addresses the first 2 aims and further research is being carried out to address the latter.
Materials and methods: People with T1DM and HU underwent semi-structured interviews. Data were recorded and analysed using grounded theory principles and interviews continued until saturation (common themes recurring).
Results: 17 people (10 female, mean age 53.1±7 yrs) were interviewed to reach saturation. The common themes were identified and categorised, this lead to the development of a theory which proposes that individuals with HU can be placed into 1 of 5 groups. Group 1: people with T1DM who may not have received adequate education about HU and do not realise that awareness can be restored. Group 2: people with HU, but they are able to carry on life quite normally with no perceived clinical or quality of life disadvantage. They do not experience severe hypoglycaemia. Group 3: those who experience horrendous episodes of severe hypoglycaemia, which they perceive as frightening, disabling and/or deeply embarrassing socially, this group evaluate and act to regain awareness. Group 4: This group all have a cognitive bias preventing them from hearing and acting upon the cues. The biases manifest in their not being able to recognise or act on the danger in which HU places them. They realise they have HU but are not 'appropriately' worried about it. The cognitive biases fall into the following categories: (a) because they do not feel any symptoms, they do not act; (b) because of fear of complications they act on any high blood glucose levels; (c) they believe 'it won't happen again'; (d) there is a strong desire to be 'normal' so may not disclose HU; (e) they believe that severe hypoglycaemia is normal, part of their lives, so nothing need be done. Group 5. those who derive some secondary gain from having severe hypoglycaemia.
Conclusion: Factors underlying the known persistence of HU in a proportion of people with T1DM can be identified for individuals and fall into one of 5 categories. Identifying the factors operating in an individual will allow therapies to restore hypoglycaemia awareness to be tailored (eg Group 1 education; Group 3 increased monitoring technology; Group 4 a psychological intervention) to their situation and help more people with T1 regain their endogenous defences against severe hypoglycaemia.
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Variable recovery from hypoglycaemic encephalopathy following massive insulin overdose in type 1 diabetes

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Background and aims: To describe and compare the clinical course of three male type 1 diabetes patients admitted to the Intensive Care Unit with hypoglycaemic encephalopathy following massive Insulin overdose, in light of available evidence.
Materials and methods: We compare outcome at 6 weeks following hypoglycaemic encephalopathy from insulin overdose and make a retrospective analysis of clinical course in the first week of admission. A review of the literature looking at the incidence, pathogenesis of hypoglycaemic encephalopathy and predictors of outcome in massive insulin overdose.

Results: Of 64 attendances for hypoglycaemia in 12 months, 3 patients (4.69%) had hypoglycaemic encephalopathy, from insulin overdose. In 2 patients the overdose was deliberate, with no previous history of depression. The patients had mean age of 37.6 years (range 32 - 41 years), with a mean capillary glucose of 2.26mmols/l (range 1.4 - 3mmol/l) at presentation. The mean interval between presentation following insulin overdose and initiation of treatment was 4.3 hours (range 1 - 8 hours). Glucagon was administered to all 3 patients and the mean glucose infused was 175g (range 50g - 425g). The mean duration of mechanical ventilation was 63 hours (range 0 - 120 hours). The outcome at 6 weeks was persistent vegetative state and death in the patient requiring maximum duration of mechanical ventilation, loss of recent memory with partial recovery at 6 weeks in the second and loss of higher cortical functions with partial recovery at 6 weeks in the third patient. The most severe outcome was in the patient requiring maximum amount of glucose and mechanical ventilation. The clinical outcome was not related to the severity of hypoglycaemia at diagnosis.
Conclusion: Hypoglycaemic encephalopathy from insulin overdose is rare. The severity of clinical outcome correlated with the interval between overdose and start of treatment, amount of glucose infused and duration of mechanical ventilation. This is in keeping with existing evidence on prognostic indicators in patients with hypoglycaemic encephalopathy and may be used to predict outcomes in acute setting.

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Detection of hypoglycaemia associated EEG-changes during sleep in type 1 diabetes

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Background and aims: Nocturnal hypoglycemia is a feared complication to insulin treated diabetes and is often the limiting factor for further intensification of treatment. Hypoglycemia unawareness, being increasingly common with tight metabolic control and long diabetes regulation, increases the risk of severe hypoglycemia. Slow wave electroencephalogram (EEG) patterns can be demonstrated during episodes of daytime hypoglycemia, and real-time detection of specific EEG changes may constitute a novel technique for a hypoglycemia alarm. Sleep EEG diverges significantly from daytime EEG including episodic occurrence of slow wave patterns. The present study tests the hypothesis that specific hypoglycemia associated EEG changes also occur during sleep, and that these can be detected in time for the patient to take action.
Materials and methods: Ten type 1 diabetes patients (mean age 47 years, diabetes duration 23,7 years, HbA1c 7.5%) all suffering from hypoglycemia unawareness, were subjected to induced hypoglycemia by graded insulin infusion both during daytime and sleep. One patient dropped out after the first night experiment. EEG was recorded from a single electrode with three measuring points placed subcutaneously at the temporal region and was analyzed real-time by an automated multi-parameter algorithm based on EEG frequency, amplitude and several other features derived from pilot experiments. The patients received an auditory alarm when EEG-changes met a predefined threshold. The patients were instructed beforehand to consume a sandwich and a juice at the time of alarm. If blood glucose fell to 1.7mmol/l without alarm, hypoglycemia was ceased. To explore intraindividual variability, the subjects were exposed to an additional night with identical study procedures but no alarm was given.
Results: Seven out of nine patients developed hypoglycemia associated EEG-changes during daytime (mean blood glucose (BG) 2.7mmol/l) of which six were able to revert hypoglycemia by carbohydrate ingestion. During sleep nine out of ten developed EEG-changes (mean BG 2,0mmol/l) and eight woke up due to the alarm. Four corrected hypoglycemia by ingestion (mean BG 2,2mmol/l) while the remaining four (mean BG 1,9mmol/l) was supplemented with glucose due to cognitive impairment. Only two events of false alarm were recorded. EEG was analyzed according to the American Academy of Sleep scoring manual to determine sleep stages. Hypoglycemia associated EEG changes occurred irrespective of the sleep stages and overruled physiological sleep related patterns.
Conclusion: We conclude that automated real-time analysis of EEG is a possible method to warn diabetes patients of impending hypoglycemia both during daytime and sleep. Post hoc improvement of the algorithm indicates that earlier detection of hypoglycemia may be possible, which will improve the sensitivity of the alarm.
Supported by: Hyposafe

PS 42 Hypoglycaemia - screening and management

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Management of hypoglycaemia in a tertiary inpatient centre: clinical audit

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Background and aims: Treatment-induced hypoglycemia causes recurrent morbidity and mortality in patients with type 1 diabetes mellitus (T1DM) and advanced type 2 diabetes mellitus (T2DM). The ACCORD study showed that patients with cardiovascular disease over an average of 3.5 years of treatment, 22% more patients from the intensively-controlled blood glucose group died. The rate of death in the intensive group, who experienced 3 times as many severe hypoglycaemic episodes compared to the standard-treatment group, is statistically significant. Profound hypoglycaemia causes neurological damage and can be fatal. In June 2009, an audit examining the identification and management of inpatient hypoglycaemic episodes was performed at St. George's Hospital. The aim of this audit was to compare current practice against good practice guidelines. Management was shown to be sub-optimal so a trustwide Hypobox and ward-based teaching sessions were commenced. A re-audit was performed to demonstrate subsequent changes and improvements in practice.
Materials and methods: 8 wards (5 medical, 1 cardiology, 2 surgical) were visited daily for one week. The diabetic charts of all patients on the ward were reviewed for episodes of hypoglycemia, how patients perceived them and how these were managed. We looked at whether efforts were made to identify the reason for the hypoglycaemic episode and whether steps were taken towards preventing a recurrence.
Results: The average age was 74 years. Mean CBG at time of hypoglycaemic episode was 3.2 mmol/L. Majority of episodes (75.6%) of hypoglycemia were mild where 95.5% had a GCS of 15/15. The remaining 24.4% of episodes were moderate (1.5 - 2.5 mmol/l). 96% of hypoglycaemic episodes were detected on routine diabetic CBG monitoring, 72% of hypoglycaemic episodes occurred between 2200 and 0600 hours. 31% of episodes were corrected according to trust guidelines appropriate for the CBG and GCS, 87.5% had their CBG rechecked. 11.1% of patients had reasons for their hypoglycaemia identified and steps were taken in 8.9 % of patients to prevent further episodes.
Comparison with the original audit: The original audit showed that only 58.3% of patients had their capillary blood glucose checked within fifteen minutes of commencing treatment for hypoglycaemia. Only 20% of patients received fast-acting carbohydrate as a treatment measure. Attempts took place to identify the cause of hypoglycaemia in one sixth of hypoglycaemic episodes, with just 12.5% of patients having steps taken to prevent the recurrence of hypoglycaemia. With the re-audit, there has been some improvement with 87% of CBGs checked post-treatment and the types of treatment used (31% from Hypobox).
Conclusion: Compared to the initial audit, improvements have been made with the products used to treat hypoglycaemia and the monitoring of patients post-treatment. Identifying and preventing the causes of hypoglycaemia remains problematic despite provision of the Hypobox and protocols. Staff awareness of hypoglycaemia guidelines is the crucial factor in ensuring patients receive optimal management and prevention of hypoglycaemic episodes. Further teaching with audit data is required.

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Fear of hypoglycaemia - how to identify patients at risk in a routine clinical practice?

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Background and aims: Hypoglycaemia is a frequent experience in people with insulin-treated diabetes mellitus and may lead to the development of fear of hypoglycaemia (FoH). This condition may be often overlooked, causing emotional and behavioral changes that can jeopardize daily life with diabetes as well as future development of diabetes complications. The aim of the study is to explore the relation of FoH to diabetes regulation, diabetes-related

problems and the awareness of hypoglycaemia in persons with type 1 (T1D) and type 2 diabetes (T2D).
Materials and methods: All insulin-treated patients ($N = 140$) registered with private diabetes centre at the time of study were contacted and 114 agreed to participate (56 males, 58 females). Age varied across sample from 15 to 88 years with a mean age of 53 (±19) years; mean ages of T1D (30 CSII and 23 MDI-treated) and T2D (61) patient subgroups were 39 years (±15) and 65 years (±13), respectively. Patients completed the Hypoglycaemia Fear Survey (HFS) and the Problem Areas in Diabetes (PAID) questionnaires. Hypoglycemia awareness status was assessed by Clarke's questionnaire. We confirmed high internal consistency reliability of the translated questionnaires (Cronbach's alphas were 0.93, 0.94, and 0.49 for HFS, PAID, and Clarke's questionnaire, respectively). Wilcoxon test (W) was used to evaluate differences between independent groups as appropriate. Metabolic control was assessed by measuring glycated haemoglobin A1c (HbA1c).
Results: Statistically significant differences across gender were found for the HFS score (Total scale: $W = 1179$, $p = 0.012$; Worry subscale: $W = 1122$, $p = 0.004$) and PAID score ($W = 1106$, $p = 0.003$), indicating greater FoH and inadequate emotional response to diabetes in females. HFS score was significantly higher in T1D in comparison to T2D patients (Total scale: $W = 2021$, $p = 0.022$; Worry subscale: $W = 2094$, $p = 0.007$). Comparing therapy regimen for T1D patients, we found significant differences for the HFS Behavior subscale score ($W = 212$, $p = 0.014$) in MDI, implying greater behavior-related FoH in MDI-treated patients. Significant correlation between HFS and PAID scores ($r = 0.70$, $p < 0.001$) indicate that FoH increases with more diabetic problems. In addition, significant correlation was found between HFS score and Clarke's score in general ($r = 0.20$, $p = 0.030$), T2D ($r = 0.27$, $p = 0.036$), T1D ($r = 0.17$, $p = 0.217$), meaning that patients with T2D experience an increase in FoH as their awareness decreases. Bivariate correlations with questionnaire scores and HbA1c level found significant association of HbA1c with HFS score ($r = 0.23$, $p = 0.015$) and PAID score ($r = 0.47$, $p < 0.001$), indicating worse glucose control with increasing FoH and diabetes problems. On the contrary, four patients had very high PAID and HFS score and low HbA1c.
Conclusion: In particular MDI-treated women with T1D, bad glycaemic regulation and lower awareness of hypoglycaemia need clinical attention, focused on hypoglycaemia. Patients with excellent glycaemic control, combined with great FoH and pronounced diabetes-related problems however, should not be overlooked.

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Nature of association between severe hypoglycaemia and risks of vascular events and death in ADVANCE

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Background and aims: The present analyses were performed to explore the risk factors for severe hypoglycaemia associated with glucose control and to examine the relationship between severe hypoglycaemia and major clinical outcomes in patients with type 2 diabetes participating in the Action in Diabetes and Vascular disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial.
Materials and methods: Baseline factors predicting severe hypoglycaemia were assessed. Associations between severe hypoglycaemia and major macrovascular or microvascular events and death were examined with Cox proportional hazards models, adjusting for baseline and post randomization covariates.
Results: Over a median of 5-years of follow-up, 231 patients (2.1%) experienced severe hypoglycaemia, a considerably lower rate than reported in other large-scale trials of intensive glucose control. Baseline factors associated with severe hypoglycaemia were age, diabetes duration, history of microvascular disease, kidney dysfunction, smoking, BMI, HbA1c, education level, cognitive dysfunction, combination anti-hyperglycaemic therapy and assignment to intensive glucose control (all $p < 0.05$). The median times from the first severe hypoglycaemia to first major macrovascular event, microvascular event or death were 1.56 years (IQR = 0.84, 2.41 years), 0.99 years (IQR = 0.40, 2.17 years) and 1.05 years (IQR = 0.34, 2.41 years) respectively. During follow up, adjusted risks of major macrovascular events (HR 2.88, 95% CI 2.01-4.12), major microvascular events (HR 1.81, 95% CI 1.19-2.74), cardiovascular death (HR 2.68, 95% CI 1.72-4.19) and all-cause death (HR 2.69, 95% CI 1.97-3.67) were significantly increased among those experiencing prior severe hypoglycaemia compared to those who did not (all $p < 0.0001$). Similar associations were also apparent for a range of non-vascular outcomes including respiratory, digestive system and skin events (all $p < 0.01$). When the analyses were performed stratified by treatment allocation, the results remained essentially unchanged.
Conclusion: Severe hypoglycaemia was strongly associated with increased risks of diverse major clinical outcomes, but not temporally related. Severe hypoglycaemia appears to be a marker of vulnerability for rather than a direct cause of adverse clinical outcomes. The complex interplay of confounding factors makes attribution of causation difficult.
Supported by: NHMRC and Servier

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The effect of modafinil on counterregulatory and cognitive responses to experimental hypoglycaemia in type 1 diabetic patients with hypoglycaemia unawareness

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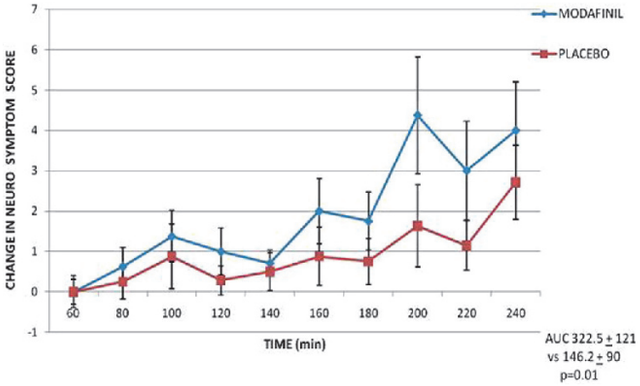
Background and aims: Patients with Type 1 diabetes rely on symptoms of hypoglycaemia to detect hypoglycaemia and take corrective action before blood glucose drops low enough to impair cognitive function or consciousness. Repeated hypoglycaemia can impair hypoglycaemia awareness increasing the risk of severe hypoglycaemia 3-6 fold. Modafinil reduces the release of the inhibitory brain neurotransmitter GABA and improves adrenergic sensitivity and some aspects of cognitive function at hypoglycaemia in normal individuals. We aimed to assess the effect of modafinil on cognitive function as well as symptomatic and hormonal responses to hypoglycaemia.
Material and methods: Eight individuals with type 1 diabetes and hypoglycaemia unawareness (mean age 47+10 years, BMI 24.3+2.5 kg/m2, HbA1c 6.6+0.9, duration of diabetes 29+12.3 years) received, in random order, two 100-mg doses of modafinil or placebo, followed by a paired hypoglycaemic clamp study in which plasma glucose was reduced stepwise to 5.0, 4.4, 3.8, 3.4, 2.8 and 2.4mmol/l. Catecholamines, symptom scores and cognitive function were measured throughout. Subjects rated neuroglycopenic (difficulty speaking, confusion, dizziness, irritability, blurred vision and drowsiness) and autonomic symptoms (sweating, anxiety, tremor, palpitations, feeling hot and tingling) on a visual analogue scale on which patients graded the symptoms from 1 (absent) to 7 (very severe) at 20 minutes intervals. Four-choice reaction time, Digit Symbol Substitution Test, Stroop Black-White, Colour-X reading and Colour-Word interference test were administered at each step.
Results: Neuroglycopenic symptoms of hypoglycaemia were experienced at higher glucose level with modafinil [mean 2.7+0.6 vs 2.3+0.4 mmol/l; p=0.025], and to a greater degree [area under curve for neuroglycopenic symptoms corrected for baseline 322.5+121 vs 146.2+90; p=0.01] (see figure) with a higher peak score [peak-baseline neuroglycopenic symptom score 4.12+3.3 vs 2.62+2.5; p=0.009]. There were no differences in autonomic symptom responses. There were no differences in glucose thresholds, area under the curve or peak levels for catecholamine responses to hypoglycaemia. Despite higher neuroglycopenic scores there was no difference in glucose thresholds or degree of deterioration in cognitive function tests.
Conclusion: The use of modafinil was associated with generation of warning symptoms of hypoglycaemia at higher glucose level and to a greater degree than placebo although there was no difference in hormonal responses or cognitive dysfunction. The data support a role for disinhibition of GABAergic neurons in the generation of neuroglycopenic symptoms in hypoglycaemia which may be reversible, but imply a different sensitivity or involvement of other pathways in other aspects of the normal counterregulatory response.



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Risk of hypoglycaemias and hypoglycaemia prevention behaving in type 1 diabetic patients - results of continuous glucose monitoring and Gravelling´s questionaire - a pilot study

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Background and aims: Hypoglycemic episode during driving could cause a fatal incident. Using a continuous glucose monitoring in type 1 diabetic patients we wanted to determine the glycemic excursions during periods of driving.
Materials and methods: We monitored 12 patients with type 1 diabetes mellitus treated with intensified insulin regimen (7 men, 5 woman), duration of disease 11,2 ± 5,2 year. Each patient wore a CGMS for 3-5 working days during his normal activity and was not alowed to see actual glycaemic values. Patients were asked to record all important events (such as insulin injection, exercise, meals, working periods) including periods of a car driving. After CMGS use, continuous glucose profiles were reviewed to identify glycaemic excursion during periods of car driving with a special interest in hypoglycaemic episodes (values under 3,5 mmol/l) and periods of glycemias under 4,5 mmol/l with considerable risk of hypoglycaemia. Each patient complete Gravelling´s questionnaire.
Results: We evaluated 2772 min (46 hours 12 min) of driving, an average 77 ± 27,3 per day and patient. Patients recorded 2 symptomatic episodes while driving. We found 7 episodes of asymptomatic hypoglyceamias, all in 2 patients. Total duration of period with glycaemia under 4,5 was 132 min (4,8% of total driving time).Total duration of period with glycemia under 3,5 was 22 min (0,8% of total driving time). Selected results from questionaire: 2 patients almost never measure glycemia before or during driving, none of them stops after hypoglycemia for longer than 45 min.
Conclusion: Risk of hypoglycaemia even in well experienced patients with type 1 diabetes mellitus during driving is considerable and should be regularly focused in education.

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Prevalence and risk factors of hypoglycaemia unawareness and severe hypoglycaemia in patients with type 1 diabetes

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Background and aims: Hypoglycaemia is the most frequent adverse effect of insulin therapy in patients with type 1 diabetes (T1DM). Repeated episodes of hypoglycaemia may lead to hypoglycaemia unawareness (HU), which in turn is a risk factor for severe hypoglycaemia (SH). Modern insulin analogues and insulin pump therapy, which are associated with lower rates of hypoglycaemia, are being increasingly used. We therefore aimed to assess the prevalence of HU and SH in a contemporary cohort of T1DM patients and to identify potential risk factors.
Materials and methods: We performed a cross-sectional study of a cohort of 486 T1DM patients (47% male), who visited the outpatient clinic of a Dutch University Hospital between 2006 and 2008. All patients were asked to complete a Dutch translation of the Clarke questionnaire, where a score of 3 or more (out of 5) was assumed to indicate HU. SH was assessed on the basis of the same questionnaire. Clinical data on demographics, diabetes treatment, concurrent medication and vascular complications were retrieved from medical records. Unadjusted and adjusted Odds ratio's (OR) were calculated using binary logistic regression.
Results: The mean diabetes duration was 25 years, HbA1c averaged 7.9% and 89% used insulin analogues. 144 patients (30%) used insulin pumps. A total of 158 patients (33%) had a score indicating HU and 103 patients (21%) recalled SH in the year prior to the questionnaire. In unadjusted analyses, HU was associated with male sex, lower HbA1c, duration of diabetes, autonomic neuropathy and estimated GFR < 60ml/min/1.73m2 (all P < 0.05). After adjustments, duration of diabetes (OR per year 1.03; 95% CI: 1.01-1.05), estimated GFR < 60ml/min/1.73m2 (3.30; 1.20-9.10) and lower HbA1c (per % 1.49; 1.22-1.81) were still associated with HU. SH was independently associated with the presence of autonomic neuropathy (3.62; 1.65-7.94) and the use of benzodiazepines (4.59; 1.80-11.73), but not with HbA1c or diabetes duration. The use of insulin analogues, insulin pump therapy, ACE inhibitors or beta-blockers were not associated with either HU or SH.
Conclusion: HU is still highly prevalent in T1DM patients despite advances in insulin therapy. Diabetes duration, lower HbA1c level and kidney dysfunction were independent risk factors for HU. Autonomic neuropathy and use of benzodiazepines were risk factors for SH. Clinicians treating patients with T1DM should be aware of the still high prevalence of HU and its risk factors.

Odds ratio for hypoglycaemia unawareness and severe hypoglycemia

	Hypoglycaemia unawareness (HU)	Hypoglycaemia unawareness (HU)	Severe hypoglycaemia (SH)	Severe hypoglycaemia (SH)
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Sex (male vs. female)	1.47 (1.00-2.15)	1.38 (0.92-2.07)	1.05 (0.68-1.62)	
Age (per year)	1.02 (1.01-1.04)	1.01 (0.99-1.03)	1.00 (0.98-1.01)	
HbA1c (per 1% lower)	1.40 (1.16-1.68)	1.49 (1.22-1.81)	1.08 (0.89-1.30)	
Diabetes duration (per year)	1.03 (1.02-1.05)	1.03 (1.01-1.05)	1.01 (0.99-1.02)	
Autonomic Neuropathy (yes vs. no)	2.34 (1.10-4.97)	1.66 (0.72-3.84)	3.27 (1.52-7.05)	3.62 (1.65-7.94)
eGFR < 60ml/min/1.73m2) (yes vs. no)	3.70 (1.43-9.59)	3.30 (1.20-9.10)	2.22 (0.85-5.79)	
Peripheral arterial disease (yes vs. no)	1.54 (0.69-3.43)		2.43 (1.07-5.54)	1.56 (0.63-3.84)
Benzodiazepine use (yes vs. no)	2.35 (0.94-5.91)		4.40 (1.74-11.13)	4.59 (1.80-11.73)

PS 43 Metabolic effects of drugs - pilot studies

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Effects of short-term continuous subcutaneous insulin infusion on insulin sensitivity and plasma FGF-21 levels in patients with new-onset type 2 diabetes mellitus

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Background and aims: FGF-21 is a recently described member of the FGF family that is highly expressed in adult mouse liver and thymus. Recent data have shown that FGF-21 is a potent regulator of glucose homeostasis. In the present study, we investigate the effects of short-term continuous subcutaneous insulin infusion(CSII) on insulin sensitivity and plasma FGF-21levels in patients with new-onset type 2 diabetes mellitus (T2DM). We also assessed the association between plasma FGF-21 body composition, and several metabolic parameters in these subjects.
Materials and methods: 30 patients with new-onset T2DM were treated with CSII for 2 weeks. A hyperinsulinemic-Euglycemic clamp was performed for determining insulin sensitivity in T2DM patients. The body composition was assesses. Blood samples were drawn after an overnight fast and Plasma insulin, FFA, HbA1c, TG, TC, LDL-C, HDL-C were measured. Plasma FGF-21 levels were measured with a radioimmunoassay. The homeostasis model assessment of insulin resistance (HOMAIR) and the homeostasis model assessment of β-cell insulin secretion (HOMAIS) were calculated. Plasma FGF-21 levels were measured by an ELISA.
Results: Fasting plasma FGF-21 levels were higher in T2DM than in controls (1.6±0.1 *vs.*1.1±0.4μg/L, P<0.01). After treatment with CSII, fasting blood glucose (FBG), HbA1c**,** fasting plasma insulin(FIns)and HOMA-$_{IR}$ were decreased significantly in T2DM (14.5±1.4 *vs.* 5.8±0.5 mmol/L, 10.4±1.2%*vs.*8.6±1.2%,16.79±2.75*vs.*12.87±5.71mU/L and 10.56±1.37 *vs.* 3.33±1.61 respectively, P<0.05 and P<0.01), while glucose infusion rate (GIR)was increased significantly (2.99±1.43 *vs.* 5.10±1.78 mg.kg^{-1}.min^{-1}, P<0.01). Fasting plasma FGF-21 levels were decreased significantly by CSII treatment (1.6±0.1 *vs.*1.3±0.1μg/L, P < 0.05).
Conclusion: Short-term CSII therapy can remarkably ameliorate insulin sensitivity and degrade plasma FGF-21 levels in T2DM patients. The change of plasma FGF-21 levels may be related to metabolic disturbance and insulin resistance.
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Comparison of fasting apelin and visfatin levels between subjects with type 1 diabetes mellitus and controls and response after insulin administration

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Background and aims: Apelin and visfatin are two recently described adipokines. Experimental data have shown that apelin exerts important beneficial cardiovascular effects and its synthesis is stimulated by insulin. Visfatin is involved in the regulation of glucose homeostasis by binding to and activating the insulin receptor in a manner distinct from insulin. No data exist on differences in plasma concentrations of apelin and visfatin between subjects with type 1 diabetes mellitus (T1DM), a condition characterized by insulin deficiency, and healthy controls and on the effect of exogenous insulin administration on plasma concentrations of these adipokines. In the present study we examined differences in plasma apelin and visfatin concentrations between healthy controls and subjects with T1DM. Additionally, we studied the effect of a bolus insulin administration on plasma apelin and visfatin levels during an oral glucose tolerance test (OGTT) in patients with T1DM.
Materials and methods: A total of 120 subjects (60 with T1DM and 60 age, gender and body mass index matched-controls) were studied. Plasma apelin, visfatin, glucose and lipids were measured in the fasting state. A total of 14 subjects with T1DM and 14 controls volunteered to participate in a second phase of the study. They were studied on two occasions (phases A and B) with a time interval of about 1 week in between and in random order. In both phases an OGTT was performed. In phase A, before the OGTT 7 units of insulin lispro (approximately 1 unit for every 10 g of glucose consumed) was administered subcutaneously in the patients with T1DM, while an equal volume of water for injection (placebo) was administered in the control group. In phase B, no insulin or placebo was administered in either patients with T1DM or controls. Plasma levels of glucose, insulin, apelin and visfatin were measured at baseline, and 10, 20, 30, 60, 90, 120, 150, and 180 min after glucose consumption.
Results: Fasting plasma apelin concentrations were significantly higher in subjects with T1DM than in controls (1.93±0.14 vs. 1.39±0.09 ng/ml, p<0.001). On the contrary fasting visfatin levels were significantly lower in subjects with T1DM than in controls (17.55±1.68 vs. 25.28±3.63 ng/ml, p<0.001). Plasma apelin and visfatin levels did not change significantly during phase A of the experiment in either the group of patients with T1DM or the controls and there was no significant difference between the two groups. The same was valid for the phase B of the study. The overall apelin and visfatin response during the experiment, expressed as the area under the curve (AUC), was not significantly different between patients with T1DM and controls during either phase A or phase B of the study.
Conclusion: Apelin levels are higher and visfatin levels are lower in the fasting state in patients with T1DM in comparison with healthy controls. Additionally, they do not change during an OGTT with or without insulin administration.

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Valsartan improves beta cell function and insulin sensitivity in normotensive subjects with impaired fasting glucose and/or impaired glucose tolerance

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Background and aims: Recently, the NAVIGATOR trial, a long-term intervention study performed in normotensive subjects with impaired glucose metabolism (IGM), showed that treatment with the angiotensin-receptor blocker valsartan (VAL) for 5 years, resulted in a relative reduction of 14% in the incidence of T2DM. The underlying mechanisms are incompletely understood and may, besides improvement in insulin sensitivity also include a delay in beta-cell function decline.
Materials and methods: In the present study, we assessed the effect of 26-weeks VAL (320mg QD) vs. placebo (PLB) on various aspects of beta-cell function and insulin sensitivity in normotensive subjects with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). In this randomized, controlled, double-blind, two-center study, 43 IFG (51% males; mean±SE age 58±1.1 yrs; BMI 28.8±0.7 kg/m^2; BP 128/82±2/1 mmHg) and 36 IFG/IGT (53% males; age 58±1.1 yrs; BMI 29.5±0.7 kg/m^2; BP 131/82±2/1 mmHg) Caucasian subjects, received VAL (n=40) or PLB (n=39). Beta-cell function and insulin sensitivity were assessed at baseline and after 26 weeks treatment using a combined euglycaemic-hyperinsulinaemic and hyperglycaemic clamp with subsequent arginine-stimulation and a 2-hour 75-g oral glucose tolerance test (OGTT). Treatment effects were analyzed using ANCOVA, adjusting for center, glucometabolic status and gender.
Results: At week 26, VAL vs PLB, increased 1st-phase (P=0.06), and 2nd-phase glucose-stimulated insulin secretion (P=0.026). However, the enhanced arginine-stimulated insulin secretion did not differ (P=0.25). VAL vs PLB improved the disposition index (P=0.067) and insulin sensitivity (P=0.045). VAL but not PLB increased the OGTT derived insulinogenic index ((I_{30}-I_0)/(G_{30}-G_0), representing 1st-phase insulin secretion after an oral glucose load, P=0.035). At 26 weeks, VAL compared to PLB treatment resulted in a significantly greater reduction in systolic and diastolic blood pressure (P<0.001). BMI remained unchanged in both treatment groups.
Conclusion: Twenty-six week VAL treatment showed a significant improvement in glucose-stimulated insulin release and insulin sensitivity in normotensive subjects with IGM. These findings may partly unveil the mechanisms underlying the observed effects of VAL in the prevention or delay of the onset of T2DM.
Supported by: An Investigator-Initiated Study Grant from Novartis Pharma

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Valsartan induced improvement of insulin sensitivity was not associated with improved microvascular function in subjects with impaired glucose metabolism

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Background and aims: Microvascular dysfunction, affecting both flow resistance and perfusion, is not only important in the development of target-organ damage in the heart, kidney and eyes, but also in the development of insulin resistance. Capillary recruitment is an important mechanism by which insulin promotes uptake of glucose. Capillary rarefaction and impaired recruitment may therefore reduce glucose uptake and contribute to insulin resistance. Recently, in the NAVIGATOR trial, a long-term intervention study performed in normotensive subjects with impaired glucose metabolism (IGM), a relative risk reduction of 14% in the incidence of T2DM was reached with the angiotensin-receptor blocker valsartan (VAL). The underlying mechanisms are incompletely understood but may include enhanced peripheral insulin sensitivity and beneficial effects on microvascular function. In this study we investigated the relation between functional and structural capillary density and insulin sensitivity in subjects with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). Furthermore, we examined whether 26-weeks of VAL treatment improved insulin sensitivity and microvascular function.
Materials and methods: Caucasian subjects with IFG (n=29, 52% males; mean±SE age 57±1.4 yrs; BMI 29±1.1 kg/m^2; BP 130/81±3/1 mmHg) and combined IFG/IGT (n=19, 63% males; mean±SE age 56±1.4 yrs; BMI 30±1.2 kg/m^2; BP 131/84±3/2 mmHg), were treated with VAL for 26 weeks. Glucose tolerance was assessed with a 2-hour oral glucose tolerance test (OGTT). At baseline and after 26-weeks subjects underwent a) an euglycaemic-hyperinsulinaemic clamp to assess insulin sensitivity and b) capillaroscopy to examine functional skin capillary density at baseline and after 4 minutes of arterial occlusion as well as structural capillary density during venous occlusion. 16 normoglycaemic healthy subjects (NGT, 62% males; mean±SE age 54±1.8 yrs; BMI 28±0.7 kg/m^2; BP 122/78±2/2 mmHg) were the control group.
Results: Subjects with IFG and combined IFG/IGT were more insulin resistant compared to NGT (M-value: 4.8 ±2.3 mg/min/kg, 4.6 ±1.2 mg/min/kg and 9.4 ±3.0 mg/min/kg, P<0.001, IFG/IGT, IFG and NGT, respectively). In subjects with IFG, IFG/IGT, capillary density was reduced in the basal state, during venous occlusion and after arterial occlusion compared to controls (all P<0.001). Functional and structural capillary density was positively correlated with insulin sensitivity (all P=0.004). In multivariable regression analyses, the associations between capillary density and insulin sensitivity were independent of age, sex, blood pressure and BMI (P=0.04). 26-weeks of VAL treatment improved insulin sensitivity (P=0.038), without changes in microvascular function.
Conclusion: Subjects with IFG and IFG/IGT are characterized by impaired functional and structural capillary density. The microvascular dysfunction was associated with diminished insulin sensitivity. Treatment with VAL did improve insulin sensitivity, however there were no changes in microvascular function.
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Effect of the interleukin-1 receptor antagonist anakinra on insulin sensitivity in obese, insulin resistant individuals: results from a double-blind placebo-controlled study

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Background and aims: The global prevalence of obesity is rapidly rising and paralleled by an increasing incidence of type 2 diabetes mellitus. Strong evidence has emerged that inflammatory changes link excess energy/fat to insulin resistance, one of the prime pathophysiological defects in type 2 diabetes. In vivo studies in mice and in vitro experiments have demonstrated that obesity activates Interleukin (IL)-1, which subsequently leads to insulin resistance. Blocking IL-1 by recombinant IL-1Receptor antagonist (Ra) thus should diminish insulin resistance. Recent findings show that blocking IL-1 by recombinant human IL-1Ra, anakinra, improves glycemic control in patients with type 2 diabetes. In the present study we investigated whether anakinra was able to improve insulin sensitivity in obese, insulin resistant but non-diabetic subjects.
Materials and methods: Non-diabetic subjects were treated with anakinra 150 mg s.c. once daily or matching placebo for 4 weeks in a double blind cross-over study. Between treatment periods there was a 4 weeks wash-out interval. Inclusion criteria: age ≥18 yrs, BMI ≥ 30 kg/m^2 and ≥ 3 characteristics of the metabolic syndrome (IDF definition). 19 Patients were included in the study and randomized. Power calculations revealed that 12 paired observations would provide sufficient power to detect a 20% difference in insulin sensitivity.Insulin sensitivity (primary endpoint) was evaluated using a euglycemic hyperinsulinemic clamp, beta-cell functions by oral glucose tolerance test (OGTT). After each treatment period, a subcutaneous fat biopsy was taken to evaluate effects at the level of the fat tissue.
Results: A total of 13 subjects (F:M = 9:4) completed all studies, 6 withdrew from the study. Most subjects experienced local injection site reactions. Average BMI at baseline was 33 ± 5 kg/m^2 and this did not change during the study. Anakinra had no effect on insulin sensitivity (glucose disposal rate 19.8 ± 2.3 for anakinra vs 18.6 ± 2.5 µMol/kg/min for placebo P=0.44, nor on insulin sensitivity index 0.17 ± 0.03 vs 0.14 ± 0.02 µMol/kg/min/mE P=0.15). HbA1c, fasting glucose levels and C-Peptide during OGTT were not affected. The disposition index improved significantly after anakinra treatment (154 ± 26 vs,106 ± 20 pmol/mmol P=0.04). Markers of systemic inflammation HsCRP, IL-6 and IL-8 remained unchanged. IL-1Ra levels in serum and in fat were significantly higher during treatment with anakinra (serum 735 ± 86 vs 0.66 ± 0.09 µg/l P<0.001, fat 217 ± 19 vs 47.7 ± 9.0 ng/mg fat tissue $P<0.001$).
Histochemical analysis of fatbiopsies showed higher numbers of CD68-positive cells, while expression of PPARγ, and FABP4 significantly decreased in adipose tissue after anakinra treatment (both P<0.05), adiponectin tended to decrease (P=0.07).No carry-over effects were found.
Conclusion: Treatment with anakinra does not result in improved insulin sensitivity in obese, insulin-resistant, non-diabetic subjects. While there was a hint towards an improved insulin secretion, no beneficial changes were found at the fat tissue level. Either the dose of anakinra is too low, the half life too short, or IL-1 plays a relatively minor role at the level of the human fat tissue.
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Long-term effect of anti TNF-alpha therapy on insulin resistance, body composition and adipokines

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Background and aims: Insulin resistance (IR) is a condition associated, among others to abdominal obesity, endothelial dysfunction, hypertension, atherogenic lipid profile, type2 diabetes mellitus and cardiovascular (CV) events. Remarkably, IR has been observed in inflammatory chronic diseases, like rheumatoid arthritis (RA), which suggests that IR and systemic inflammatory response are linked events. It has been suggested that tumour necrosis factor(TNF)-α, a proinflammatory cytokine that plays a key role in the pathogenesis of several human inflammatory disease, might be a connection between inflammation and CV disease apparently through its effects on endothelial function, vascular homoeostasis, IR, as well as on production of adipokines. However, the clinical significance of this potential association has not been fully confirmed. The aim of this study was to determine whether or not chronic blockade of systemic TNF-α in humans has some influence on CV-associated risk factors, such as insulin sensitivity, body fat distribution, body composition, physical activity or levels of adipokines.
Material and methods: Sixteen patients with RA (mean age 50.8±14.6 years, mean duration of disease 6.3±2.7 years) in whom anti-TNFα agents were added to methotrexate because active disease were followed up during a year. At baseline and after 3 and 12 months of TNFα treatment, we assessed: disease activity by DAS28, physical activity by accelerometry, anthropometric measures, IR by Homeostatic Model Assessment (HOMA-2), body composition by multifrequency bioelectric impedance analysis, abdominal fat dis-

tribution (subcutaneous and intraabdominal) by magnetic resonance (MR) imaging and serum level of several key adipokines by ELISA. None patient used steroids a month before the start or during the study.
Results: In spite of a significant improvement in DAS28, patients' physical activity remains stable during the follow up. The body mass index showed a significant increase after one year (25.7±3.2 vs 28.06±4.5 kg/m2, p=0.02) of treatment with anti-TNFα. The body composition (impedance) in terms of fat and fat-free mass showed no difference between visits except for a significant elevation of body cell mass (25.5±4.6 vs 26.6±3.1 kg, p=0.02). Values of visceral intraabdominal and subcutaneous abdominal adipose tissue by MR were not modified because of treatment. HOMA-2 values showed not changes in β-cell function or IR index but a significant increase (110[94-138] vs 118[107-156]%, p=0.045) in insulin sensitivity after 3 months was observed. Basal levels of adiponectin, visfatin, leptin, ghrelin, resistin, and apelin did not change in response to anti-TNF-α treatment; only retinol binding protein 4 showed a significant change (51.7±32.7 vs 64.9±28.4 μg/mL, p=0.03) at the end of the study.
Conclusions: IR, adiposity, body composition and adipokines are not significantly affected by 1 year blockade of TNFα in RA patients. More studies with longer period of follow-up must be done to further clarify the real beneficial role of chronic anti-TNFα treatments in cardiovascular risk factors.
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In type 2 diabetic patients the improvement in insulin sensitivity is associated with a decrease in plasma C-peptide levels and an improvement in intima media thickness
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Background and aims: There is increasing evidence that in type 2 diabetic patients increased C-Peptide levels might predict vascular inflammation and the progression atherosclerotic plaques. The aim of this study was to investigate a potential relationship between the decrease in plasma C-peptide levels and an improvement in intima media thickness (IMT) during treatment with Pioglitazone.
Materials and methods: The Pioneer Study investigated the change in IMT over 6 months in 89 type 2 diabetic patients treated with pioglitazone (PIO: age 62.2±8.4 years; duration of diabetes 89.8 months) and in 84 type 2 diabetic patients treated with glimepiride (GLIM: age: 63.0±7.4 years; duration of diabetes 82.5±77.5 months). At baseline and after 6 months the IMT was measured at the common carotid artery and fasting blood samples were taken for the measurement of glucose, insulin, C-peptide, and HbA1c. The $HOMA_{IR}$ Score was calculated as a measure of insulin resistance.
Results: Both treatments resulted in a comparable improvement in HbA1c levels (PIO: from 7.52±0.85 to 6.71±0.89; GLIM: from 7.44±0.89 to 6.83±0.85 %, p<0.05). During treatment with pioglitazone the IMT declined from 0.949±0.149 to 0.893±0.144 mm (p<0.05), while no significant change in IMT could be observed in the GLIM group (from 0.924±0.150 auf 0.911± 0.158 mm (n.s.). Pioglitazone treatment declined the $HOMA_{IR}$ Score from 6.2±4.1 to 3.9±1.9(p<0.05), and C-peptide levels from 2.03±0.94 to 1.69±0.75 ng/ml (p<0.05) . During GLIM treatment, $HOMA_{IR}$ changed from 5.8±3.7 to 6.0±4.7 (n.s.), and C-peptide changed from 1.95±0-85 to 1.94±0.96 ng/ml; (n.s.). A linear correlation could be observed in between the decrease in IMT and the $HOMA_{IR}$ Score (r=0.29; p<0.05), and a decrease in plasma C-peptide (r=0.28; p<0-05).
Conclusion: This study confirmed a linear association between the decrease in IMT and an improvement in insulin sensitivity, as well as a decline in plasma C-peptide levels. The study further emphasizes the complex interrelationship in between Insulin and C-peptide secretion and vascular pathology in patients with diabetes mellitus type 2.
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Long-term treatment with the angiotensin-receptor blocker valsartan improves adipose tissue function in normotensive subjects with impaired glucose metabolism
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Background and aims: Adipose tissue dysfunction may contribute to the development of obesity-related insulin resistance and type 2 diabetes (T2D). Increased activity of the renin-angiotensin system in obese insulin resistant subjects may be implicated. The recently published prospective NAVIGATOR trial demonstrated that the angiotensin-receptor blocker valsartan (VAL), at median follow-up 5.0 years, reduced the incidence of T2D by 14% compared to placebo (PLB) in normotensive subjects with impaired glucose metabolism (IGM). However, no underlying mechanisms were reported. We hypothesized that VAL treatment in subjects with IGM improves adipose tissue function, which in turn may contribute to increased insulin sensitivity.
Materials and methods: In this randomized, placebo-controlled, double-blind, two-center study, the effect of VAL (320 mg daily) or PLB on insulin sensitivity (hyperinsulinaemic-euglycaemic clamp) was investigated before and at 26 weeks of therapy in 79 normotensive subjects with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) (52M/48F; age 58±7 yrs; BMI 29.7±4.3 kg/m^2; BP 130/82±11/6 mmHg). In a subgroup of 17 IFG (10M/7F; age 60±1 yrs; BMI 29.5±0.9kg/m^2; BP 127/78±2/3 mmHg) and 14 IFG/IGT (6M/8F; age 59±1 yrs; BMI 32.1±1.2kg/m^2; BP 129/82±2/2 mmHg) subjects matched for age, BMI and blood pressure, the effects of VAL or PLB on adipose tissue function was examined. Adipose tissue blood flow (ATBF) was measured under fasting conditions and for 4h after intake of a high-fat mixed-meal using the ^{133}Xe wash-out technique. In addition, abdominal subcutaneous adipose tissue biopsies were obtained for measurement of mean adipocyte diameter and size distribution. In addition to treatment effects, baseline comparisons were made between men and women and between subjects with different glucometabolic status (mixed ANOVA).
Results: At baseline, fasting ATBF was lower (P=0.01) and mean abdominal adipocyte size higher (increase in fraction of large adipocytes and decrease in small adipocytes, P<0.01 for both) in women (7IFG / 8IFG/IGT) compared to men (10IFG / 6IFG/IGT). No significant differences were found for baseline ATBF and adipocyte size between IFG and IFG/IGT subjects. VAL increased insulin sensitivity (P=0.045), fasting ATBF (P=0.02) and postprandial $ATBF_{AUC\ 0\text{-}4h}$ (P=0.04) compared to PLB. In addition, mean adipocyte diameter was decreased by VAL compared to PLB (P<0.001), with a shift towards a higher proportion of small and less large adipocytes (P<0.001 for both), whereas no changes in body weight were noted.
Conclusion: 26-weeks VAL treatment increased insulin sensitivity, fasting and postprandial ATBF and decreased mean abdominal subcutaneous adipocyte size, with a shift from large to small adipocytes, in subjects with IGM. The present data suggest that improved adipose tissue function may contribute to the reduced development of T2D in patients following long-term VAL treatment.
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The relationship of serum A-FABP, retinol binding protein 4, and adiponectin levels to the development of metabolic syndrome in children: a 3-year prospective cohort study

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Background and aims: Adipocyte-fatty acid binding protein (A-FABP) and retinol binding protein 4 (RBP4) has been reported to be associated with metabolic syndrome in adults. We evaluated the prospective association of A-FABP, RBP4, and adiponectin with metabolic syndrome as defined by pediatric adaption of the National Cholesterol Education Program criteria.
Materials and methods: In this prospective cohort study, 159 boys from the school-based Metabolic disorders & Obesity Study in Elementary School children (MOSES) study were followed up for 3 years.
Results: At baseline, A-FABP levels (12.8 ± 5.1 μg/L vs. 23.6 ± 8.2 μg/L, *P* <0.001) and RBP4 levels (59.7 ± 15.3 vs. 69.3 ± 17.1, *P* = 0.001) were significantly higher, whereas adiponectin levels (18.1 ± 8.4 μg/mL vs. 11.5 ± 5.4 μg/mL, *P* <0.001) were significantly lower in overweight children compared to normal control. During 3 year of follow-up, in addition to their association with change of weight, A-FABP levels were positively associated with change of waist circumference and adiponectin levels were negatively associated with change of triglyceride. Although both baseline A-FABP and adiponectin were associated with the development of metabolic syndrome, multiple logistic regression analysis showed that only A-FABP was the independent predictor of the development of metabolic syndrome after adjustment for Tanner stage, insulin resistance, and BMI during 3 year of follow-up (odds ratio, 12.0; 95% CI, 1.3 to 107.4; highest versus lowest tertile).
Conclusion: A-FABP predicts the development of metabolic syndrome independently of adiposity and insulin resistance in Korean boys.

Table 1. Logistic regression analysis of baseline adipokines in the prediction of the development of metabolic syndrome at 3 years later.

		Q1	Q2	Q3
Adiponectin		2.42-11.04	11.19-18.47	18.66-41.08
	Model 1	1.0	0.10 (0.01-1.04)	0.07 (0.01-0.73)
	Model 2	1.0	0.03 (0.01-0.87)	0.09 (0.01-0.6)
	Model 3	1.0	0.31 (0.03-3.47)	0.12 (0.01-1.51)
A-FABP		5.0-11.0	12.0-17.0	18.0-43.0
	Model 1	1.0	1.09 (0.06-18.81)	10.82 (1.15-101.52)
	Model 2	1.0	1.02 (0.06-16.79)	9.56 (1.15-79.64)
	Model 3	1.0	1.29 (0.08-21.47)	12.00 (1.34-107.36)

Model 1: adjusted for Tanner stage
Model 2: adjusted for model 1 plus HOMA-IR at baseline
Model 3: adjusted for model 2 plus BMI at baseline

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The relationship between liver fat content and insulin resistance and beta cell function in individuals with different status of glucose metabolism

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Aims: Nonalcoholic fatty liver disease (NAFLD) is commonly associated with type 2 diabetes, dyslipidemia, and insulin resistance. Many studies have shown NAFLD was a predictor of type 2 diabetes and metabolic syndrome. Excess deposition of liver fat may be affect the glucose by deteriorating the β-cell function and increasing the insulin resistance. This study aims to study the relationship between liver fat content(LFC), insulin resistance and β-cell function in individuals with different status of glucose metabolism in China.
Materials and methods: 109 subjects including 31 impaired glucose regulation (IGR), 31 newly diagnosed type 2 diabetes (NT2DM) and 47 normal control(NC) with normal metabolic parameters were involved in the study by measuring the LFC using Proton magnetic resonance spectroscopy, evaluating the insulin resistance and β-cell function using C peptide and insulin from oral 75g glucose tolerance test.
Results: (1)LFC was 3.83%, (inter-quartile range, 2.35-7.59%),12.82%(inter-quartile range, 8.10-21.37%),21.99%(inter-quartile range, 11.89-34.43%) in NC,IGR,NT2DM groups and was elevated in turn(*P*<0.01);(2) The subjects were divided into four subgroups by LFC Quartile named Quartile 1-4 associated with the increasing LFC.HOMA-IR were elevated from Quartile 2 by turns(*P*<0.01);(3) Insulin from 0 to 30 min (ΔI_{30}),the ratio of change in insulin from 0 to 30 min to that in glucose from 0 to 30 min ($\Delta I_{30}/\Delta BG_{30}$),and C peptide from 0 to 30 min (ΔCP_{30}) increased in Quartile 2,then decrease in Quartile 3,but without statistical significance. In Quartile 4 ,$\Delta I_{30}/\Delta BG_{30}$ and ΔCP_{30} sharply decreased(*P*<0.05,and *P*<0.01). The ratio of C peptide from 0 to 30 min to that in glucose from 0 to 30 min ($\Delta CP_{30}/\Delta BG_{30}$) begun to decrease from Quartile 3(*P*<0.05). The ratio of area under curve of C peptide to area under curve of glucose (CP_{AUC} / BG_{AUC}) were significantly decreased from Quartile 3(*P*<0.05). From Quartile 3,glucose level abnormally elevated to the diagnosis value of IGR(*P*<0.01)(Tab 1);(4) LFC was positively correlated with HOMA-IR(r_s =0.618)(*P* <0.01),but was negatively correlated with ΔCP_{30}(r_s=-0.282),$\Delta CP_{30}/\Delta BG_{30}$($r_s$=-0.404),$CP_{AUC}$ / BG_{AUC}(r_s=-0.308)(All *P* <0.01);(5)Stepwise regression analysis demonstrated LFC was the strongest predictor of HOMA-IR .But LFC failed to enter all models about β-cell function.
Conclusion: LFC was an independent risk factor of insulin resistance. When LFC moderate increased, the early phase of insulin secretion also compensatively increased, but as the LFC further deposition, both the early phase and whole β-cell function were deteriorated, and hyperglycemia developed. These suggest that deposition of LFC participates in the pathogenesis of type 2 diabetes.

Tab 1 Comparison of Insulin Resistance and β-cell function among quartile of LFC values

	Q1	Q2	Q3	Q4	*F* value
$\lg\Delta I_{30}$(mU/L*min)	2.7±0.3	2.8±0.3	2.8±0.4	2.7±0.4	0.50
ΔCP_{30} (ng/ml*min)	38.4±15.5	38.8±13.5	33.3±14.9	27.6±15.1†§	0.01
$\lg\Delta I_{30}/\Delta BG_{30}$ (mU/L/mmol/L)	1.0±0.4	1.1±0.4	0.9±0.5	0.8±0.4‡	0.07
$\Delta CP_{30}/\Delta BG_{30}$ (ng/ml/mmol/L)	0.8±2.1	0.7±1.8	0.5±2.0*‡	0.3±2.6†§¶	0.00
$\lg Ins_{AUC}/BG_{AUC}$ (mU/L/mmol/L)	0.8±0.3	0.8±0.2	0.8±0.2	0.7±0.3	0.44
CP_{AUC}/BG_{AUC} (ng/ml/mmol/L)	0.5±0.2	0.5±0.2	0.4±0.2*	0.4±0.2 †‡	0.01
HOMA-IR	1.9±1.5	2.8±1.7†	4.0±1.7†§	4.9±1.6†§	0.00

*F <0.05, †F <0.01, vs.Q1; ‡F <0.05, §F <0.01, vs.Q2; ¶F <0.05, vs.Q3

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Defects in glutaeal adipogenesis are associated with obesity-related insulin resistance in black South African women

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Background and aims: It has been proposed that defects in the ability to store fat in subcutaneous adipose tissue (SAT) depots may be associated with increased visceral adipose tissue (VAT) accumulation and decreased insulin sensitivity (S_I). The aims of the study were to measure the expression of genes involved in adipogenesis in abdominal SAT and gluteal depots, and determined their relationship with VAT and S_I in black and white South African women who differed in body fat distribution and S_I.
Materials and methods: VAT (computerised tomography scan), S_I (frequently sampled intravenous glucose tolerance test), and abdominal and gluteal SAT gene expression were measured in 14 normal-weight (BMI <25 kg/m^2) black, 14 normal-weight white, 15 obese (BMI >30 kg/m^2) black and 13 obese white South African women.
Results: With increasing BMI, black women accumulated less VAT (P=0.03) and more abdominal SAT (P=0.017), but had lower S_I (P<0.01) than white women. After adjusting for differences in VAT and S_I, abdominal SAT adipogenic gene expression did not differ by ethnicity. However, gluteal SAT expression of adipogenic transcription factors (CEBPβ and STAT5a) were

higher in black than white women (P<0.05). In contrast, gluteal expression of adipogenic (PPARγ), lipogenic (FASN, PEPCK) and lipolytic (HSL, FABP) genes were down-regulated in obese black women (P<0.05). Further, CEBPα (r=0.53, P=0.007), FASN (r=0.50, P=0.011), PEPCK (r=0.45, P=0.024), LPL (0.52, P=0.008), HSL (r=0.59, P=0.002) and FABP (r=0.61, P=0.001) mRNA levels in gluteal SAT, but not abdominal SAT, correlated with S_I in black women, independent of % body fat. In white women, FASN expression in both gluteal and superficial SAT depots correlated equally with S_I (r=0.47, P=0.025). VAT correlated negatively with mRNA levels of adipogenic transcription factors (CEBPα (r=-0.47, P=0.025), CEBPβ (r=-0.44, P=0.037) and SREBPS (r=-0.46, P=0.028)) in white women, whereas in black women, no associations with VAT were found.
Conclusion: Obese black women have impaired expression of gluteal SAT adipogenic genes compared to white women, which associates with reduced S_I. These findings therefore refute the hypothesis that black South African women display 'healthy obesity' due to their greater peripheral fat distribution, but rather suggests that obesity in black women impairs gluteal SAT adipogenesis and storage, potentially leading to insulin resistance, which increases the risk for type 2 diabetes.
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Adipose tissue dysregulation and reduced insulin sensitivity in non-obese individuals with enlarged abdominal adipose cells
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Background and aims: Obesity promotes type 2 diabetes through induction of insulin resistance. Insulin resistance in the adipose tissue is, however, seen long before type 2 diabetes develops and this is associated with a dysfunctional adipose tissue including an impaired ability to recruit pre-adipocytes and, thus, promoting adipocyte hypertrophy. Animal studies have shown that expansion of the adipose tissue does not necessarily affect the metabolic phenotype as long as the adipose tissue is not dysfunctional. In order to examine this in man, we here investigated GLUT4 expression and other markers of insulin resistance in the adipose tissue, including adipose cell size as a marker of impaired pre-adipocyte differentiation in non-obese, first-degree relatives to type 2 diabetic patients in relation to the whole-body phenotype.
Materials and methods: 34 non-obese, first-degree relatives to type 2 diabetic patients were recruited through advertisement in a local newspaper. Anthropometric measures were recorded, blood samples collected for quantification of circulating factors and insulin sensitivity measured by the hyperinsulinaemic-euglycaemic clamp method. Abdominal subcutaneous adipose tissue biopsies were obtained, adipocyte cell size and gene and protein expression were measured.
Results: Our findings show that these individuals, although non-obese, exhibit clear signs of a dysfunctional adipose tissue, characterized by low expression of GLUT4, altered adipokine profile, and enlarged adipocyte cell size that correlate with the clinical phenotype and insulin sensitivity. In summary, cell size, in these non-obese individuals, is correlated with WHR (R=0.42, p=0.014), f-glucose (R=0.30, p=0.085), fs-insulin (R=0.58, p<0.001), HbA1c (R=0.33, p=0.058), GIR (R=-0.41, p=0.016), TG (R=0.44, p=0.009), s-HDL cholesterol (R=-0.41, p=0.017), s-Adiponectin (R=-0.56, p=0.001), GLUT4 mRNA (R=-0.45, p=0.007) and GLUT4 protein (R=-0.29, p=0.095). In addition, GLUT4 expression in the adipose tissue and circulating adiponectin, which is secreted from the adipose tissue, was closely related (mRNA: R=0.42, p=0.013; protein: R=0.36, p=0.037) and show a similar association with clinical parameters.
Conclusion: In conclusion, these findings support the concept that it is not obesity per se, but rather the presence of a dysregulated adipose tissue which relates to whole-body insulin sensitivity and the associated phenotype.
Supported by: SRC Novo Nordisk SDF SSF Söderberg Wiberg Bergvall

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Retinol binding protein-4, insulin sensitivity and intima media thickness in non diabetic subjects with normal glucose tolerance
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Background and aims: Retinol binding protein-4 (RBP4) is a novel adipokine able to modulate the action of insulin in several tissues; its role in humans is controversial. RBP4 has been associated to increase in carotid intima-media thickness (IMT) in hypertensive women but its association with early atherosclerotic changes in normortensive subjects was not studied. Thus, the aim of the present study was to evaluate the relationship between RBP4 and insulin sensitivity and IMT in lean and obese non-diabetic normotensive subjects.
Materials and methods: Insulin sensitivity (euglycemic hyperinsulinemic clamp), IMT in common carotid artery (high-resolution B-mode ultrasound) and serum RBP4 levels were assessed in 72 subjects (39 males, 43.9 ± 8.3 years; BMI 27.1 ± 4.6 kg/m2 [range 19.1 to 30.3 kg/m2]). Study subjects were divided in lean (L, n=29), overweight (OW, n=17) and obese (OB, n=26).
Results: Insulin sensitivity (M value) ranged from 11.9 to 81.2 μmol/kg FFM/min (mean±SD = 50.0±14.0 μmol/kg FFM/min) and was lower in OB (L = 60.0±12.7; OW = 45.7±11.1 and OB = 42.3±12.4μmol/kg FFM/min, p<0.0001). IMT was higher in OB and OW, as compared to L (OW = 0.601 ± 0.081 and OB = 0.658 ± 0.092mm vs. L = 0.589 ± 0.074, p=0.01). RBP4 levels were similar between genders, BMI subgroups and between the lowest and higher M quartile (56.2 ± 32 vs. 55.8 ± 28.3 μg/ml; p=ns). In the whole study no relationship was observed between RBP4 and BMI, blood pressure, M value, IMT, plasma adiponectin and glucose and insulin plasma levels during the OGTT. IMT was independently correlated only with age and weight (r2=0.35; p<0.0001).
Conclusion: In non-diabetic normotensive subjects, RBP4 levels are neither related to the degree of obesity and of insulin sensitivity nor to blood pressure and to IMT. In this healthy population increase in IMT seems to reflect physiologic aging and adaptation to body size.

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Nonalcoholic steatohepatitis (NASH) is a prediabetic state frequently associated with abnormal glucose tolerance and severe hepatic and adipose tissue insulin resistance
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Background and aims: No previous study has systematically screened patients with nonalcoholic steatohepatitis (NASH) for type 2 diabetes mellitus (T2DM). In addition, the role of hyperglycemia and insulin resistance (IR) in relation to the severity of NASH is poorly understood. To better understand this, we studied the prevalence of impaired fasting glucose (IFG), IGT and T2DM, and the impact of glucose tolerance status and IR on liver histology, in biopsy-proven NASH pts from the University of Texas at San Antonio NASH Trial (NCT00994682). This large (n=120) study aims to understand the metabolic/molecular mechanisms associated with NASH and the efficacy/safety of long-term treatment with pioglitazone.
Materials and methods: We report on 79 subjects. Clinical characteristics: age=51±1, gender=52/27 (M/F), BMI=35.8±0.9 kg/m2, FPG=123±4 mg/dl, A1c=6.4±0.2%, AST=48±3/ALT=73±3 IU/L). We measured: 1) glucose, insulin and FFA during an OGTT; 2) liver fat by magnetic resonance spectroscopy (MRS); 3)]liver/muscle (Rd) insulin sensitivity (euglycemic insulin clamp with 3H glucose and indirect calorimetry); and 4) hepatic (Hep-IR index= EGP x fasting plasma insulin [FPI]) and adipose tissue (Adipo-IR index= fasting FFA x FPI) insulin resistance. Liver histology was assessed by Kleiner criteria.
Results: In a predominantly Hispanic population (~2/3) glucose metabolism was abnormal in 90% of patients with 51% of these being unaware of this until testing. In those not known previously to have diabetes, 22% were newly diagnosed T2DM, 40% had both IFG and IGT, 20% IFG or IGT while only 18% had normal fasting/postprandial glucose metabolism. While T2DM and non-diabetics with NASH were well matched for age, ethnicity, percent body fat (DXA), lipids and liver steatosis (both by MRS [27±3%] and histology), T2DM patients had worse steatohepatitis (data available in 69) (necroinflammation= 2.8±0.1 vs. 1.8±0.1) and ALT (83±9 vs. 64±5 IU/L, both p<0.001). Diabetics were more insulin-resistant at the level of the liver (Hep-IR=36.1±9.0 vs. 23.9±4.6 mg/kg−1•min−1•μU/ml; suppression of EGP by low-dose insulin infusion: -59% vs. 83%, both p<0.001) and adipose tissue (Adipo-IR= 13.3±1.8 vs. 8.5±1.1 mmol/liter•U/ml, p<0.001). In contrast, Rd (muscle) was diminished but identical in both groups (2.3±0.4 mg/kg−1•min−1). Hepatocyte necroinflammation correlated closely with A1c, Hep-IR and Adipo-IR (p<0.05-0.01).
Conclusion: The prevalence of IFG, IGT and T2DM in a predominantly Hispanic population with NASH is much higher than previously appreciated and such patients may benefit from early screening for T2DM. Patients with NASH and T2DM have worse steatohepatitis, which appears related at least in part, to their more severe hepatic and adipose tissue insulin resistance.
Supported by: The Burrough Wellcome Fund, American Diabetes Association, VAMC and NCRR

PS 45 Cardiometabolic risk assessment

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Insulin resistance, rather than BMI, predicts metabolic severity in anorexia nervosa

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Introduction: Anorexia nervosa (AN) is a psychiatric eating disorder, characterized by self-induced weight loss and body image distortion. The prolonged starvation causes changes in body composition as loss of fat and lean masses while nutritional recovery results in significant changes in body composition, especially in fat mass distribution as well as in FT3 normalization. It is well known that changes in body composition may modify insulin-sensitivity (IS) nevertheless, the determination of insulin sensitivity in AN has provided conflicting results.
Aim: In our study we aimed to examine IS, measured by hyperinsulinemic euglycemic clamp (HEC), in patients affected by AN. The second aim was to evaluate the relationship between IS and the usual clinical parameters of recovery from AN, including FT3 and body fat mass.
Subjects and methods: 26 women with restrictive AN (age 24.4± 6.0 years; disease duration 66.4±7.8 months), were enrolled. We measured BMI, hormones (FT3, FT4, TSH, serum and urinary free cortisol, GH and IGF-1), and performed OGTT and HEC. Body composition was determined by dual energy X-ray absorptiometry (DEXA). Fat body mass was calculated for the total body and for trunk and leg regions. We considered patients in partial recovery if FT3 was within normal range (≥ 2 pg/ml).
Results: All studied subjects were normo-tolerant. After dividing the cohort based on FT3 levels, the two groups were similar in age and disease duration although subjects with normal FT3 levels had higher BMI, total fat mass, trunk fat mass and ratio trunk fat mass/total fat mass ($p<0.05$). No differences in the levels of plasma and urinary cortisol, GH, TSH, FT4 were present. The group with low FT3 had lower IGF1 ($p<0.05$). A linear negative correlation was observed between glucose uptake during HEC and trunk fat mass (R=-0.72; p=0.01), while no correlation between glucose uptake and BMI (R=-0.08; p=0.82) was found. A linear negative correlation was shown between FT3 and glucose uptake (R=-0.75; p=0.01); and (positive) with abdominal fat (R=0.901; p=0.003).
Discussion: The inverse correlation found between body fat mass (trunk and total) and glucose uptake and the absence of any correlation between BMI and glucose uptake allow us to hypothesize that, in patients with anorexia nervosa, BMI is not a reliable index of metabolic status, in opposite to what was observed in patients with metabolic syndrome or diabetes. The measurement of body fat by DEXA seems to be more reliable than BMI. Our study also showed that the metabolic alterations and the body composition were correlated with indices of disease that per se could constitute a marker of the metabolic state in these patients, offering a reliable prognostic indications. In perspective, although AN patients in partial recovery were still with an extremely reduced fat mass, we hypothesize that the reappearance of fat exclusively in the abdomen induces insulin resistance and may contribute to the usual difficulty to reach normal weight in these patients.

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Does insulin resistance or insulin secretion predict weight changes in non-diabetic subjects?

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Background and aims: Previous studies using the euglycaemic insulin clamp technique have reported that a high degree of insulin sensitivity predicts weight gain. This association, however, has not been confirmed in other studies using different surrogate measures of insulin sensitivity. Likewise, both increased and decreased insulin secretion have been linked with weight gain. We therefore undertook to systematically analyse the relationship between insulin sensitivity/secretion and spontaneous weight changes in non-diabetic subjects.
Materials and methods: In 1,048 subjects from the RISC cohort (561 women and 467 men, mean age 44 years) followed up for 3 years, we measured baseline insulin sensitivity (by a 240 pmol.min.m-2 insulin clamp) and ß-cell function (i.e., fasting insulin secretion rate, total insulin output and ß-cell glucose sensitivity, by mathematical modelling of the C-peptide response to oral glucose).
Results: In the whole cohort, both men and women gained weight over 3 years (0.9 [4.6] and 0.9 [4.6] kg, respectively, median [IQR], $p<0.0001$ vs zero). Baseline BMI was significantly higher in both weight gainers (top 20% of the distribution of BMI changes, +6 [3] kg) and weight losers (bottom 20%, -4 [3] kg) as compared to weight stable subjects (25.7 [5.0] and 26.3 [4.6] kg.m-2 , respectively, vs 24.3 [4.6] kg.m-2, $p<0.0001$ for both). In contrast, insulin sensitivity (as the M or M/I index) was not associated with either weight gain or weight loss across quartiles of baseline BMI. By multiple logistic or linear regression analyses adjusting for centre, age and sex, baseline waist circumference (or BMI or body weight) was the only significant, independent predictor of both weight gain and weight loss. In none of these models did any parameter of ß-cell function show an independent association with weight changes; this was also the case for baseline glucose tolerance status (NGT, IFG or IGT).
Conclusion: In a large cohort of non-diabetic Caucasian subjects, neither insulin sensitivity nor insulin secretion predicts spontaneous weight changes. As compared to lean subjects, heavier individuals are prone to either gaining or losing weight regardless of their degree of insulin sensitivity.
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A multi-marker risk score predicts insulin sensitivity and beta cell function-driven type 2 diabetes from a fasted sample

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Background and aims: The Insulin Resistance in Atherosclerosis (IRAS) Study demonstrated that insulin sensitivity (SI), acute insulin response (AIR) and the disposition index (DI) as measured by frequently-sampled intravenous glucose tolerance tests (FSIGT) were each predictive of future diabetes in a multi-ethnic population. While such careful measurements of glucose homeostasis provide useful prognostic information, the FSIGT test itself is impractical for standard clinical practice. The PreDx™ Diabetes Risk Score (DRS) is a prognostic test that combines the fasting concentrations of glucose, insulin, hemoglobin A1c, adiponectin, C-reactive protein, ferritin and interleukin-2 receptor alpha into an absolute risk for conversion to diabetes within five years (as defined by the WHO 1999 criteria).
Materials and methods: We measured the DRS in baseline IRAS samples from 722 subjects (including 127 that subsequently converted to diabetes) and evaluated the correlation of the DRS and its individual components with SI, AIR and DI. To further evaluate the relationship of the DRS to insulin sensitivity, insulin secretion or beta cell function, we determined the incremental performance of logistic models combining SI, AIR or DI with the fixed DRS score in predicting incident diabetes.
Results: The DRS was strongly and significantly correlated with both SI ($r = -0.52$, $p < 0.01$) and DI ($r = -0.54$, $p < 0.01$), and weakly correlated with AIR ($r = -0.20$, $p < 0.01$). Additionally, each of the individual components of the DRS except IL-2 receptor alpha correlated significantly with SI and DI. The DRS, SI, AIR and DI were each significant predictors of the onset of diabetes ($p < 0.01$) with C-statistics of 0.75, 0.68, 0.69 and 0.79, respectively. Adding SI to DRS did not significantly improve the prediction of incident diabetes, indicating that the DRS captures the biological basis of SI-induced diabetes. Conversely, adding DRS increased the C-statistics of SI by 0.07 ($p < 0.01$) and AIR by 0.10 ($p < 0.01$), but did not improve the performance of the DI.
Conclusion: The DRS correlates strongly and significantly with SI and the DI. In addition to this correlation the DRS substitutes for, and exceeds, the capacity of SI to predict incident diabetes ($p < 0.01$), and it can be performed from a fasted sample. The DRS represents a simple and practical approach to identifying patients at risk for SI or DI-driven type 2 diabetes.

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Screening for insulin resistance in persons with increased diabetes risk by EZSCAN

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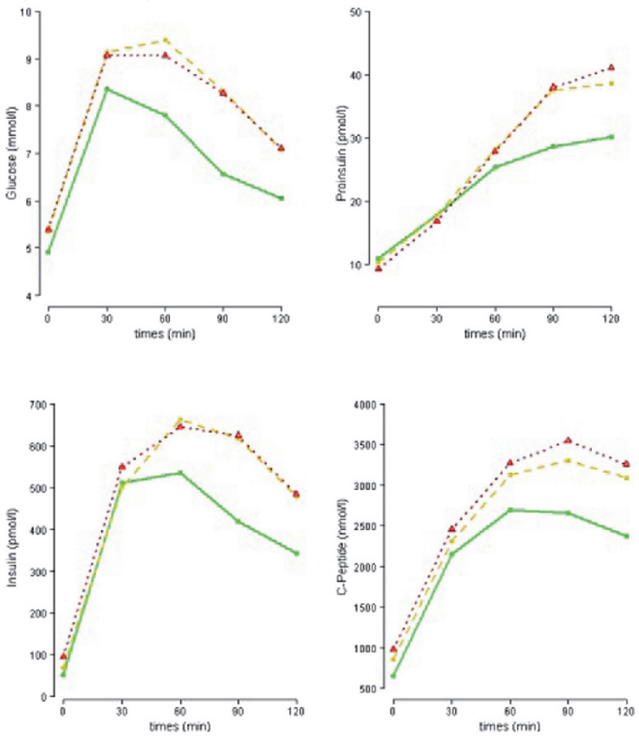
Background and aims: Prevalence of type 2 diabetes and pre-diabetes are increasing globally. Early identification and intervention even in persons with pre-diabetes will reduce/delay progression to diabetes and related complications. There is a lack of simple, fast, reproducible and cheap methods for early diagnosis and screening of people at risk for diabetes. EZSCAN was recently developed to measure sweat gland function through measurement of electro skin conductance (ESC) based on reverse iontophoresis. The aim of the study was to evaluate the ability of EZSCAN to predict insulin resistance in subjects at high risk of diabetes.
Materials and methods: 230 subjects with a family history of type 2 diabetes, obesity or dyslipoproteinaemia from the city of Dresden and adjoining area (37% male, mean age 56.4±13.9yrs, mean BMI 27.8±5.0kg/m2) were involved in the study and had an oral glucose tolerance test (OGTT) with measurement of plasma glucose, pro-insulin, insulin and C-peptide at 0, 30, 60, 90 and 120 minutes. EZSCAN results are given in colour codes based on the values of ESC on feet, hands and forehead: Green - Normal; Yellow - Borderline; Orange-Red - High risk.
Results: Based on ESC values measured by EZSCAN 56 subjects were classified as green, 115 as yellow and 54 as orange-red. Results of glucose, proinsulin, insulin and C-peptide for OGTT according to EZSCAN classification are displayed on Figure. Patients classified as green on EZSCAN had significant lower values for glucose and insulin at 60, 90 and 120 min. and for C-peptide at 90 and 120 min. when compared to patients of yellow or orange-red groups.
Conclusion: EZSCAN is a new simple and non invasive method for identifying subjects at high risk for diabetes.



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Serum advanced glycation end products is associated with insulin resistance independent of adiponectin

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Background and aims: Recent in vitro experimental data have suggested that advanced glycation end products (AGEs) may play a significant role in the development of insulin resistance by interfering with the molecular pathways of insulin signaling. We have investigated whether the degree of insulin resistance in human subjects is associated with circulating level of AGEs.
Materials and methods: 165 healthy non-diabetic subjects (77 male and 88 female) were recruited from the community. Serum levels of AGEs and adiponectin were measured by ELISA. Insulin resistance was determined by using the homeostasis model assessment index (HOMA-IR).
Results: Serum AGEs was higher in male than female subjects (3.91 ± 1.18 unit/ml vs 3.49 ± 1.22, p=0.03). There was no significant difference in age between male and female subjects but male subjects had higher body mass index (25.5 ± 2.6 kg/m^2 vs 23.6 ± 3.3, p<0.01) and waist circumference (86.4 ± 7.1 cm vs 76.4 ± 8.6, p<0.01). Male subjects were more insulin resistant, HOMA- IR [median 1.37 (interquartile range 0.81 - 2.19) vs 1.12 (0.64 - 1.78), p=0.04] and had lower serum adiponectin level [6.24 ug/ml (3.99 - 8.98) vs 10.67 (7.92 - 15.12), p<0.01]. In both male and female subjects, serum AGEs correlated with HOMA-IR (r=0.37, p< 0.01; r=0.26, p=0.02 respectively). Serum AGEs remained an independent determinant of HOAM-IR even after adjusting for age, gender, waist, smoking and adiponectin level on multiple linear regression analysis.
Conclusion: Formation and accumulation of AGEs progress during normal aging. We have demonstrated that circulating level of AGEs is associated with insulin resistance in non-diabetic subjects independent of adiponectin.

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Alzheimer's disease in normoglycaemic patients: an association with decreased insulin sensitivity and atherogenic profile of lipid abnormalities

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Background and aims: Previous studies have suggested that insulin resistance might play an important role in pathogenesis of Alzheimer's disease (AD), but the relevant mechanisms of this influence have not yet been clarified. The study was aimed to analyze in patients with AD the levels of (a) insulin sensitivity (IS), (b) plasma insulin (PI) and (c) lipid parameters comprising total cholesterol (Ch), low-density (LDL) and high density (HDL) Ch, triglycerides and apolipoproteins (apoAI, apoAII, apoB, Lp(a) and apoE) potentially involved the pathogenesis of the disease.
Materials and methods: In the study we included 57 normoglycemic patients with AD (group A; BMI: 23,95 +/- 0.81 kg/m2, mean age of 72,34+/-8,60 years) and 25 matched controls (group B; BMI: 25,43 +/- 0,66 kg/m2, mean age of 61,33+/-6,75 years). IS was evaluated by using euglycemic hyperinsulinemic clamp technique with insulin infusion rate of 1 mU/kgbw/min during 120 min and glucose infusion adjusted manually, at 5 min intervals, to maintain target euglycemia. Total glucose uptake (M value) was calculated on the basis of the amount of glucose infused during steady state period (80-120 min). PI levels were determined by radioimmunossay and PG levels by glucose oxidase method. Total cholesterol, HDL-Ch, and triglycerides levels were determined by using enzymatic method, and LDL-Ch was calculated using the formula of Friedewald. Apolipoproteins ApoAI, ApoAII, Lp(a), ApoB and ApoE were determined by using nephelometry method.
Results: We found that total glucose uptake was significantly lower in group A compared to group B (6,41 +/- 0,60 vs 8,13 +/- 0,33 mg/min/kg, p<0.01). In addition, basal PI levels were higher in group A compared to group B (15,57 +/- 2,01 vs 7,34+/-0,98 mU/l, p<0.05), while basal PG levels did not differ between the groups. Moreover, the levels of total Ch and LDL-Ch were significantly higher in group A in comparison to group B (6,47 +/- 0,23 vs 5,71 +/- 0,19; 4,40 +/- 0,19 vs 3,58 +/- 0,17 mmol/l, respectively, p<0.01), while the HDL-Ch levels were significantly lower in group A than in group B (1,21 +/- 0,04 vs 1,48 +/- 0,32 mmol/l, p<0.01). The levels of triglycerides did not differ significantly between the groups (1,58+/- 0,14 vs 1,58 +/- 0,13 mmol/l, p=NS. In addition, the levels of ApoAI were significantly lower in group A in comparison to group B (1,493+/-0,058 vs 2,02+/-0,37 g/l, p<0.01), while the

levels of other apolipoproteins, ApoAII, ApoB, Lp(a) and ApoE did not differ significantly between the groups (330,24+/-10,57 vs 330,37+/-12,07 mg/L; 1,135 +/- 0,056 vs 1,06 +/- 0,28 g/l; 0,264+/-0,053 vs 0,24+/-0,07 g/l; 42,63+/-1,89 vs 40,40+/-1,15 mg/L, respectively, p=NS).
Conclusion: Our results have demonstrated that the presence of AD in normoglycemic patients was associated with decreased IS and increases in peripheral insulin levels. Our results imply that decreased IS levels might exert, at least partly, their pathogenic influence through the lipid abnormalities especially the decreases in HDL-Ch and ApoAI levels.

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Relationship between insulin resistance and puberty in obese children with increased cardiometabolic risk
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Background and aims: Epidemiolgy data provide evidences that the frequency of obesity and cardiometabolic risk factors shows an increasing tendency in childhood. Insulin resistance playa a central role in the pathogenesis of cardiovascular and metabolic consequences of obesity. Transient decrease in the insulin sensitivity during puberty is a well-known physiological process, however the feature of this phenomenon is not clear in obese children with increased cardiometabolic risk. The aim of present study was to assess the effect of puberty on insulin resistance and metabolic parameters in obese children with and without increased cardiometabolic risk.
Materials and methods: Anthropometry data, insulin levels during OGTT and lipid status were analyzed of 161 obese children aged 10-18 years. Σinsulin/Σglucose ratio obtained during glucose load and HOMA index were used to assess insulin resistance. Children were sorted into prepubertal (T1), pubertal (T2-4) and postpubertal (T5) cohorts according to Tanner staging criteria and metabolic and insulin resistance parameters were evaluated. Increased cardiometabolic risk (CMR) was defined as the presence of any two risk factors (elevated FPG, BP, TG or decreased HDL-C) in addition to obesity.
Results: Out of 161 obese subjects, 43 (26.7%) had CMR. Decreased HDL and/or elevated TG was observed in 92 (57.1%) cases. IGT and/or IFG was found in 25 (15.5%) cases. In subjects without CMR, the Σinsulin/Σglucose ratio in T1 stage was significantly lower than in T2-4 and T5 stages (p=0.01). In children with CMR, the Σinsulin/Σglucose ratio was similar in T1, T2-4 and T5 stages, however it was significantly higher in T1 stage as compared to subjects without CMR (p=0.04). In T2-4 and T5 stages, the Σinsulin/Σglucose ratio did not differ between children with and without CMR. No difference was found in HOMA index between groups with and without CMR in T1 stage, however significantly higher levels were observed in CMR subjects in T2-4 stages (p=0.01), indicating the presence of fasting hyperinsulinaemia in this cohort. Elevated HbA1c (>6.0%) was found in 13 (12.6%) out of 81 children investigated, of whom only two cases had abnormal OGTT results. In cases having normal HbA1c, OGTT showed IGT in 2 cases, IFG in 2 cases IGF and IGT together in 2 cases and T2DM also in 2 cases, respectively.
Conclusion: Increased insulin resistance can be observed in obese children without CMR. In obese children with CMR, substantial insulin resistance occurs in prepuberty and it is present in similar level throughout puberty. Fasting insulin levels are elevated in obese CMR subjects as compared to those without CMR. To reveal T2DM cases, HbA1c and OGTT results should be assessed in parallel.

PS 46 At home with HOMA?

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***In vivo* glucose and amino acid sensitivity to insulin in subjects with impaired glucose regulation**
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Background and aims: Insulin is central to control of glycaemia but also regulates tissue protein metabolism including skeletal muscle, a major contributor to post-prandial glucose disposal.
Hypothesis: A dysregulation in insulin sensitivity to glucose will be accompanied by a comparable change in sensitivity of protein metabolism in subjects with impaired glucose regulation (IGR) as has been shown in healthy volunteers by Chevalier et al. This change in insulin sensitivity may occur through suppression of endogenous glucose production (EGP) and protein breakdown and/or altered disposal of glucose and amino acids (AA).
Materials and methods: Twenty-four volunteers (16 men, 8 women; mean age 60.3 years (52-68 years)) with IGR had assessment of basal EGP and whole body protein breakdown determined with stable isotope tracers. This was followed immediately by a 3h hyperinsulinaemic-euglycaemic-euaminoacidaemic clamp (40 mU/m^2/min) to assess insulin sensitivity. During the clamp, suppression of EGP and whole body protein breakdown were determined with the stable isotopes. The relationship between basal metabolic parameters and both glucose and AA disposal with other variables were investigated using regression analyses.
Results: Mean pre-clamp EGP was 2.80mg/kgFFM/min (2.08-4.40 mg/kgFFM/min) with a mean suppression of 78.8% (63.6-98.2%) during the clamp and mean protein breakdown was 5.20 mg/kgFFM/min (4.20-8.06 mg/kgFFM/min) with suppression of 30.1% (15.0-50.7%) during the clamp. Mean disposal of exogenous glucose was 5.63 mg/kgFFM/min (Range: 1.85-13.30 mg/kgFFM/min) and mean disposal of total glucose (exogenous glucose and residual EGP) was 6.12 mg/kgFFM/min (Range: 2.34-14.07 mg/kgFFM/min). Mean disposal of exogenous AA was 1.13 mg/kgFFM/min (Range: 0.49-1.76 mg/kgFFM/min) and mean disposal of total AA (exogenous and non-suppressed protein breakdown) was 4.72mg/kgFFM/min (Range: 3.05-5.94 mg/kgFFM/min). Exogenous glucose disposal (EGD) did not show a strong relationship with exogenous AA disposal (R^2=0.14, NS), nor was there a relationship between total glucose and AA disposal. EGD and total glucose disposal showed inverse relationships with HOMA-IR (R^2=0.38, P=0.008; R^2=0.35, P=0.01), weight (R^2=0.37, P= 0.002; R^2=0.34, P=0.003), 2 hour venous glucose (75g OGTT) (R^2=0.33, P=0.009; R^2=0.32, P=0.004) and fasting insulin (R^2=0.40, P=0.007; R^2=0.37, P=0.009). A relationship was demonstrated between total AA disposal and HOMA-IR (R^2=0.34, P=0.008). AA disposal (total or exogenous) was not related to other variables studied.
Conclusion: The hyperinsulinaemic-euglycaemic clamp is a well recognised tool in the assessment of insulin sensitivity; use of an AA clamp is less common. Pereira et al found that HOMA-IR predicted 44% of the variance in the AA clamp in men with type 2 diabetes. Whilst we found similar results between total AA disposal and HOMA-IR, on direct comparison during the insulin clamp no relationship was seen between AA disposal and glucose disposal. These findings in subjects with IGR contrast with Chevalier et al who found a correlation between glucose and AA disposal during the hyperinsulinaemic-euglycaemic-euaminoacidaemic clamps in healthy volunteers.

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Mesenteric fat thickness explains the inconsistent relationships between central obesity and insulin resistance
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Background and aims: Waist circumference is a screening tool to identify high risk individuals for insulin resistance. However, some obese subjects are insulin sensitive while some non-obese subjects exhibit features of insulin resistance. We previously reported that mesenteric fat thickness (MFT) measured by ultrasound scan explained most of the variance of cardiometabolic

risks. Here, we hypothesize that MFT may explain the inconsistent relationships between central obesity (CO) and insulin resistance.
Materials and methods: This is a cross-sectional study of MFT by ultrasound on 68 healthy Chinese men (mean age [±SD]: 43.7 ± 7.7 years, median: 44.5 years, range 26-68 years). High MFT was defined as MFT ≥ mean ±1 SD (8.7 + 2.9 = 11.6 mm) and CO as WC ≥85 cm.
Results: In these 68 men, 35 (51.5%) did not have CO (CO-) and 33 (48.5%) had CO (CO+). In the CO+ group, subjects in the top quartile of HOMA-IS had lower MFT, higher adiponectin level and were less likely to have fatty liver than the less insulin-sensitive subjects. In the CO- group, subjects in the top HOMA-IR quartile were more likely to have fatty liver, higher MFT and lower adiponectin level than the less insulin resistant subjects. Stratified by CO and MFT, 7 (21.2%) had (CO+MFT+), 26 (78.8%) had (CO+MFT-), 5 (14.3%) had (CO-MTF+) and 30 (85.7%) had (CO-MTF-). The CO+MF+ (1.31 ± 0.51) and CO-MF+ (1.34 ± 1.55) groups had the highest HOMA-IR followed by the CO+MF- (1.17 ± 0.57) and CO-MFT- (0.79 ± 0.37) groups (p-value for trend = 0.036). On multivariate analysis, the independent predictors for HOMA-IS were age, BMI and MFT.
Conclusion: The heterogeneous relationships between IR and CO are largely attributed to MFT which can be used to identify non-obese but insulin resistant subjects as well as obese but insulin sensitive subjects.

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Prevalence, metabolic features and prognosis of metabolically healthy obese Italian individuals: the Cremona Study
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Background and aims: Some obese individuals have normal insulin sensitivity and appear metabolically healthy. Whether this phenotype is associated with increased all-cause mortality risk is controversial.
Materials and methods: We established all-cause mortality through Regional Health Registry files in 2,074 Caucasian middle-aged (57±11 years), individuals of the population survey carried out in 1990-1991 in Lombardy, Italy (Cremona Study) 15 years after the baseline assessment. Study subjects were divided in four categories based on BMI (non-obese: < 30 kg/m^2; obese; ≥ 30 kg/m^2) and estimated insulin resistance (insulin-sensitive: HOMA-IR < 2.5; insulin-resistant ≥ 2.5).
Results: Data for 2,011 individuals were available. 43 of the 380 obese individuals (11%; 95%CI: 8.1-14.5%) were insulin-sensitive based on HOMA-IR. Their BMI was not different when compared to obese insulin-resistant (32±4 vs 33±3 kg/m^2), but they had lower waist circumference (94±9 vs 104±11 cm; p<0.05), blood pressure, fasting glucose, triglycerides and fibrinogen, and higher HDL-cholesterol. The total number of deaths after 15 years was 495 (CVD: 221, cancer: 180). We found that age, and sex adjusted all-cause mortality was higher in the obese insulin-resistant (HR: 1.40; 95% CI: 1.08-1.81; P=0.01) but not in the metabolically healthy obese (HR: 0.99; 95% CI: 0.46-2.11; P=0.97) when compared to non-obese insulin-sensitive subjects. Also CVD and cancer mortalities were higher in the obese insulin-resistant (HR for CVD: 1.61; 95% CI: 1.10-2.36; P=0.015 and HR for cancer: 1.52; 95% CI: 1.02-2.26; P=0.04, respectively) but not in the metabolically healthy obese (HR for CVD: 0.73; 95% CI: 0.18-3.00; P=0.66 and HR for cancer: 1.04; 95% CI: 0.32-3.30; P=0.95, respectively) when compared to non-obese insulin-sensitive subjects.
Conclusion: The metabolically healthy obese phenotype is less frequent than previously thought and in contrast with the obese insulin resistant subjects, did not show an increased all-cause mortality risk during the 15 years observational period.
Supported by: Italian Minister of Health MURST Cofin, an EFSD Clinical Research Grant

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Insulin demand, beta cell response and glucose control among contemporary children - an eight-year longitudinal study
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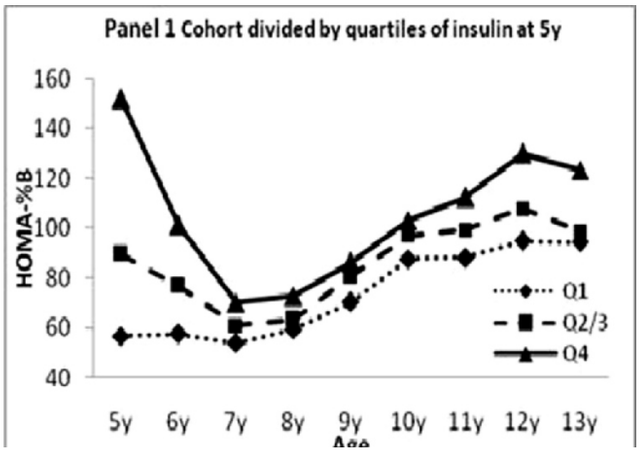
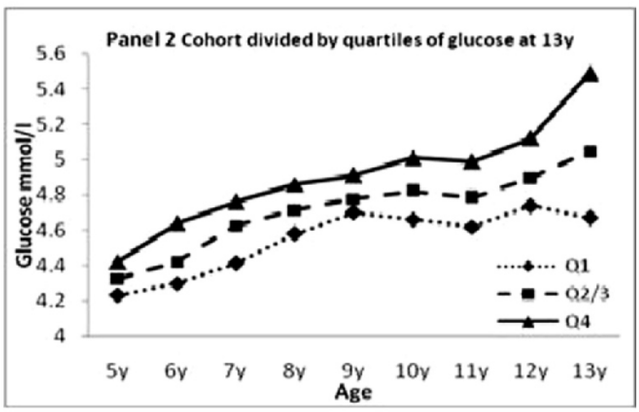
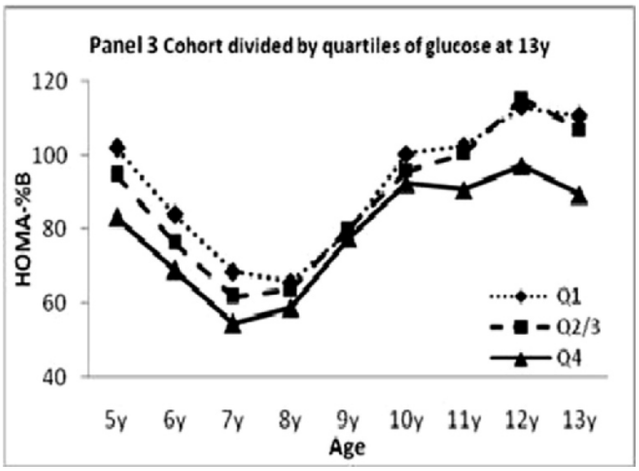
Background and aims: Glucose control becomes impaired when insulin secretion can no longer meet insulin demand. Little is known of their trends over time in contemporary children, for whom obesity, insulin demand and diabetes are rising progressively.
Materials and methods: We plotted the trajectories of fasting glucose and insulin annually over eight years in a single cohort of 258 healthy children (144 boys) and modelled the corresponding beta-cell response from the HOMA2 programme. Analyses were conducted according to exposures at 5y (quartiles of insulin and quartiles of BMI) and outcome at 13y (quartiles of glucose). The children were of uniform age (SD±3m) and race (99% White Caucasian), and were randomly selected at 5y from 54 primary schools.
Results: Mean glucose rose linearly from 4.3 mmol/l at 5y to 5.1 mmol/l at 13y (p<0.001).
Exposures: BMI had no impact on the behaviour of glucose. HOMA-B rose gently from 5-13y among children in the lowest quartile for insulin (panel 1), consistent with a progressive rise in demand, but fell steeply from 5-7y - despite rising glucose - among those in the highest quartile (consistent with a primary loss of beta cell function in those most beta cell stressed). *Outcome:* mean glucose at 13y was considerably higher in Q4 (5.5 mmol/l) than Q1 (4.7 mmol/l, p<0.001), and had been higher throughout (panel 2). Fasting insulin levels, on the other hand, were similar (at 5y Q1 4.10, Q4 3.42 mu/l, p=0.13; at 13y, Q1 6.11, Q4 7.22 mu/l, p=0.16). The difference between Q1 and Q4 children for glucose at 13y lay in HOMA-B (panel 3), which was substantially lower in Q4 than Q1 (83.1% v 102.5% at 13y, p=0.005) Gender, physical activity (accelerometry) and pubertal stage all influenced these trends, but only marginally.
Conclusion: HOMA-B should be interpreted in the context of insulin demand, and the striking difference for children in the highest quartile for glucose at 13y was not their insulin demand at 5y, but their lack of beta cell response to the subsequent rise in insulin demand - consistent with (and possibly explaining) their relatively poorer glucose control.







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Insulin resistance and increased PAI-1 as factors of non-alcoholic fat liver disease in children, adolescents and youth metabolic syndrome

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Background and aims: In metabolic syndrome (MS) patients, abdominal obesity accompanied with hyperinsulinism and insulin resistance is related to hypertension and lipid status disturbance where thrombotic and inflammatory factors and low antioxidant status and tendency to early atherosclerosis are present. Hepatic fat accumulation in childhood obesity is associated with increased visceral fat and insulin resistance (IR). IR results in fat deposition in the liver and occurrence of non-alcoholic fat liver disease (NAFLD). The study was aiming at determining NAFLD and its most important provoking factors in MS and pre-metabolic (pre-MS) syndrome patients.
Materials and methods: The study included 173 obese individuals aged 7 to 30 classified into 3 groups: I-children (7-15), II-adolescents (16-20) and III-youth (20-30). Three of the following five criteria were used for metabolic syndrome (MS) diagnosis in adolescents: waist circumference >90Pct; triglycerides >1.7mmol/l; HDL-cholesterol<1.0mmol/l; hypertension>90Pct, glycemia >6.0mmol/L. ATP III classification was applied for youth. Patients with less than three afore mentioned criteria were considered patients with pre-MS. OGTT was used to evaluate the extent of disorder. Insulin sensitivity was determined by HOMA IR. PAI-1 was determined by plasminogen substrate assay**.** SGOT, SGPT and γ-GT were considered liver function parameters. Liver ultrasonography was used to diagnose NAFLD.
Results: NAFLD, increasing considerably with age, was found in 7.3% children, 18.9% adolescents and 29.0% youth (p<0.05). NAFLD existed in 17.5% pre-MS and 29.0% MS patients. NAFLD found by groups: pre-MS patients - I-11.5%, II-17.7%, III-20.4%; MS patients - II-20.4%, III-40.0%. Logistic regression analysis indicated the most important NAFLD factors: body weight - odds ratio (OR) 1.039, p<0.001; LDL-cholesterol OR 1.55, p<0.05; creatinine clearance OR 1.01, p<0.05; uric acid OR 1.00, p<0.05; insulins - 0min OR 1.012, p<0.002, 120min OR 1.008, p<0.001; HOMA IR OR 1.059, p<0.001; PAI-l OR 2.79, p<0.001; SGPT OR 1.27, p<0.001. Patients with NAFLD had increased WC (110.7±11.9cm), LDL-cholesterol (3.3±1.0mmol/l), triglycerides (1.81±1.15mmol/l), uric acid (383.8±86.3), insulins 0min (61.1±81.3U/l) and 120min (93.1±108.4U/l), HOMA IR (14.7±4.4±19.3μmol/mU/ml), PAI-1 (7.3±0.6U/ml), SGPT (56.7±20.9U/l), γ-GT (44.1±22.8U/l). Patients without NAFLD had normal SGPT, γ-GT, uric acid and increased WC (98.6±16.7cm), insulins 0min (21.6±31.3U/l) and 120 min (44.3±49.5U/l), HOMA IR (6.2±3.4μmol/mU/ml), triglycerides (1.74±1,63mmo/l), PAI-1 (6.0±1.4U/ml) but lower than NAFLD patients.
Conclusion: Obesity, hyperinsulinemia with IR (characterized by increased uric acid and PAI-1), SGOT and LDL-cholesterol are the most frequent risk factors for NAFLD. NAFDL may be the liver sign of pre-MS and MS children, adolescents and youth associated with visceral obesity, IR, lipid status disturbance, thrombotic and inflammatory factors.

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Steatohepatitis is closely associated with insulin resistance and the metabolic syndrome from early stages of their development

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Background and aims: Non-alcoholic fatty liver disease (NFALD), insulin resistance, and the metabolic syndrome were examined for correlation in individuals undergoing elaborate health checkup programs with the influence of abdominal obesity being ruled out.
Materials and methods: Of the 909 subjects undergoing the health checkups, 626 individuals who underwent a 75 g OGTT and were evaluated by abdominal ultrasound for fatty liver and the metabolic syndrome were enrolled in the study, and 130 individuals each with fatty liver (fatty liver group; FLG) and without fatty liver (non-fatty liver group; NFLG), who were matched for gender, age, BMI, and waist circumference, were compared for relevant biochemical parameters, insulin resistance, number of risk factors implicated per individual, and frequency of the metabolic syndrome detected.
Results: There was no significant difference between the FLG and the NFLG in the male to female ratio (%), age, BMI (24.8 ± 2.9 and 24.2 ± 1.8, respectively), and waist circumference (85.8 ± 6.6 and 84.5 ± 5.8, respectively). In contrast, significantly higher values were noted in the FLG than in the NFLG with regard to the area under the glucose curve at 75 g OGTT (363.0 ± 81.3 versus 319.0 ± 70.6; $P < 0.001$), area under the insulin curve (109.0 ± 80.4 versus 76.1 ± 45.1; $P < 0.001$), HOMA-R index (1.73 ± 1.24 versus 1.17 ± 0.56; $P < 0.001$), HbA1c, AST, ALT, TG, and LDL-C, while HDL-C was significantly lower in the FLG than in the NFLG. Additionally, significantly higher values were noted in the FLG than in the NFLG with regard to the number of risk factors implicated per individual (1.83 ± 1.15 versus 1.37 ± 1.09; $P < 0.001$), frequency of the metabolic syndrome detected (30/130, 23.1% versus 14/130, 10.8%; $P < 0.05$).
Conclusion: Study results suggested that NAFLD is closely associated with insulin resistance and the metabolic syndrome even when the influence of abdominal obesity is excluded. It was further suggested that, given the BMI of < 25 kg/m^2 and the waist circumference of no more than 85 cm in the subjects, NAFLD appears to be implicated in the pathogenesis of insulin resistance and the metabolic syndrome from quite early stages of their development.

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Distinctive metabolic signature in subjects with early onset type 2 diabetes

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Subjects with early onset type 2 diabetes have severe insulin resistance, reduced VO_2 max response to exercise, and abnormal mitochondrial function relative to equally obese insulin resistant control subjects. Having previously used a metabolomics approach to demonstrate that obese insulin resistant subjects have a distinct metabolic profile compared to lean controls, we have now studied subjects with early-onset type 2 diabetes. We used targeted MS/MS and GC/MS-based metabolomics to measure fasting plasma concentrations of amino acids and total and free fatty acids in 24 subjects with early onset type 2 diabetes (mean age 26.1, BMI 35.6 kg/m2), 17 obese controls (mean age 22.8, BMI 34.2 kg/m2) and 28 lean controls (mean age 24.7, BMI 22.4 kg/m2). Confirming previous studies, the obese subjects had increased levels of branched-chain and other amino acids, total non-esterified fatty acids (NEFA), and several individual fatty acid species compared to lean controls. Interestingly, subjects with type 2 diabetes exhibited additional increases in levels of valine, leucine/isoleucine, glutamate/glutamine, and aspartate/asparagine, as well as NEFA and individual fatty acids compared to obese controls. Insulin resistance, measured by HOMA-IR correlated with concentrations of valine, leucine, histidine and glutamate.

Parameter studied (μM)	Early onset type 2	Obese controls	Lean controls	P value (1 vs. 2)	P value (1 vs. 3)
L-valine	298	261	214	0.031	<0.001
L-leucine/isoleucine	206	170	151	0.004	<0.001
L-aspartic acid/aspargine	103	78	70	0.009	<0.001
L-glutamic acid/glutamine	101	87	71	0.04	<0.001
Histidine	80	92	72	0.02	0.01
Total fatty acids	15424	13004	9520	0.039	<0.001
Palmitic acid (C16:0)	2629	2125	1442	0.021	<0.001
Oleic acid (C18:1)	4881	3703	2385	0.025	<0.001
Stearic acid (C 18:0)	41	31	25	0.012	<0.001

We conclude that subjects with early-onset type 2 diabetes have a metabolomic profile distinguishing them from BMI-matched insulin resistant individuals with normal glucose tolerance. Further studies are needed to assess whether these changes are a reflection of altered mitochondrial function in these subjects.

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Insulin resistance is associated with metabolic syndrome but not with angiographically determined coronary artery disease in female patients

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Background and aims: Insulin resistance (IR) is the key feature of the metabolic syndrome (MetS) and in prospective studies predicts atherothrombotic events. Its association with directly visualised coronary atherosclerosis, especially in female patients, is unclear. We hypothesised that IR is associated with both angiographically determined coronary artery disease (CAD) and with the MetS.
Material and methods: We enrolled 354 consecutive female patients undergoing coronary angiography for the evaluation of suspected or established stable CAD; significant CAD was diagnosed in the presence of significant coronary stenoses with lumen narrowing ≥50%. IR was determined by the HOMA index; the MetS was defined according to ATPIII criteria.
Results: HOMA-IR scores were significantly higher in MetS female patients than in female subjects without the MetS (4.9 ± 4.7 vs. 1.9 ± 1.1; p <0.001). In contrast HOMA-IR did not differ significantly between patients with significant CAD and those who did not have significant CAD 3.3 ± 3 vs. 3.1 ± 3; p = 0.823). When both, the presence of MetS and of significant CAD were considered, HOMA-IR was significantly higher in patients with the MetS both among those who had significant CAD (4.9 ± 4.8 vs. 1.9 ± 1.1; p <0.001) and among those who did not have significant CAD (5.0 ± 4.7 vs. 1.9 ± 1.1; p <0.001) whereas it did not differ significantly between patients with significant CAD and subjects without significant CAD in patients with the MetS (5.0 ± 4.7 vs. 4.9 ± 4.8; p = 0.383) nor in those without MetS (1.9 ± 1.1 vs. 1.9 ± 1.0; p = 0.860). Similar results were obtained with the IDF definition of the metabolic syndrome.
Conclusion: In female patients IR is significantly associated with the MetS but not with angiographically determined coronary atherosclerosis.

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Obstructive sleep apnoea and metabolic abnormalities in type 2 diabetes

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Background and aims: The burgeoning load of type 2 diabetes is a major public health concern in our part of the world with high morbidity, mortality, and health-care costs. Recent reports have indicated that the majority of patients with type 2 diabetes also have obstructive sleep apnea (OSA). There is compelling evidence that OSA is a significant risk factor for cardiovascular disease and mortality. Because both diabetes and OSA are associated with increased cardiovascular morbidity and mortality, it is possible that the presence of both conditions results in additive or even synergistic health risks. The aim of this study was to evaluate the prevalence of OSA in the study population and its effect on the metabolic profile.
Materials and methods: After taking the informed consent of the subjects, we performed polysomnography studies in 30 consecutive patients with diabetes and obesity according to the Asian-Indian criteria recruited from outpatient clinics between July 2009 and January 2010. Apnoea- hypopnoea index (AHI) > or = 10/hour was considered relevant for OSA diagnosis. Subjects with AHI< 10 were considered as controls. We assessed AHI, Epworth sleepiness scale (ESS), body mass index (BMI, kg/m^2), glycosylated haemoglobin (HbA1c, %), fasting serum total cholesterol (mg%), HDL-(mg%), LDL-cholesterol(mg%), triglycerides (TG) (mg%), HOMA index and highly sensitive C-reactive protein (hsCRP, mg/l).
Results: Data are presented as mean +/- SD or median (interquartile range) for parametric and nonparametric data respectively. 22 out of 30 subjects (73%) of with diabetes had OSA (AHI > or =10). AHI in the OSA group was 21 (16-30) and 5 (3-8) in controls (p < 0.001). BMI was higher in OSA (33.8 +/- 5.8) vs. controls (29.4 +/- 3.1) (p = NS). Patients with OSA had higher HbA1c (9.72 +/- 0.9) vs. (8.94 +/- 0.8)(p = 0.03), TG (210 +/- 55.2) vs. (140.2 +/- 41.9) (p = 0.046), HOMA-IR (2.35 +/- 1.6) vs. (1.93 +/- 1.5)(p = 0.046) and hsCRP (4.2 +/- 0.9) vs. (2.89 +/- 1.4)(p = 0.01). HDL-cholesterol was lower in OSA group compared to control (30.8 +/- 6.1 vs. 40.3 +/- 11.4) (p = 0.02). HbA1c correlated best with AHI(p < 0.001, r = 0.39).
Conclusion: Identifying the possibility of previously unrecognized OSA amongst patients with diabetes is important for treating physicians even in the absence of specific symptoms. The high prevalence of OSA in obese patients with type 2 diabetes is also associated with more severe metabolic derangements and its treatment along with adjustment of antidiabetic therapy may ameliorate some of the associated morbidity and mortality.

PS 47 Liver metabolism

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High dietary fat consumption decreases hepatic glucokinase activity and net hepatic glucose uptake in the absence of impaired insulin signalling in dogs

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Background and aims: High dietary fat consumption has been associated with a reduction in insulin's ability to suppress hepatic glucose production during euglycemia, but its impact on net hepatic glucose uptake (NHGU) and disposition during hyperglycemia (HG) has not been determined. The purpose of the present study was to elucidate the effect of high fat feeding on the ability of the liver to take up glucose in the presence of hyperinsulinemia (HI), HG and the portal glucose signal (PS).
Materials and methods: Adult male dogs were fed either a high fat diet (HFD, % kcal: 53% fat, 21% protein, 26% carbohydrate; *n*=4) or a chow control diet (CTR, % kcal: 26% fat, 31% protein, 43% carbohydrate; *n*=5) for 4 weeks. Dogs underwent hepatic/portal vein catheterization at 2 weeks and an HIHG clamp 2 weeks later. Somatostatin was infused peripherally (Pe) to disable the endocrine pancreas while glucagon (basal) and insulin (4x basal) were replaced intraportally (Po). The glucose load to the liver was doubled first by infusing glucose Pe (Period 1; P1), then by infusing glucose Po (PS; 22.2 μmol/kg/min) and Pe (Period 2; P2).
Results: When challenged with HI and HG (P1), NHGU (μmol/kg/min) was significantly lower in the HFD group (0.0±0.0.5) compared to the CTR group (9.4±1.1, $P<0.01$). Consistent with this finding, glycogen synthesis (μmol/kg/min; HFD: -1.4±0.3 [breakdown], CTR: 4.0±1.7, $P<0.01$) and net hepatic lactate output (μmol/kg/min; HFD: 2.9±0.4, CTR: 6.8±0.9, $P<0.01$) were also significantly lower in the HFD group compared to the CTR group. On the other hand, Po glucose delivery (PS; P2), elicited a comparable increase in NHGU (ΔP1-P2, μmol/kg/min; HFD: 8.2±1.7, CTR: 9.0±2.6, $P=0.7$) and glycogen synthesis (ΔP1-P2, μmol/kg/min; HFD: 9.0±2.7, CTR: 10.9±2.5, $P=0.8$) in both groups, suggesting that the portal glucose signal was still effective in the HFD group. Terminal liver biopsies revealed a reduction (≈20%) in glucokinase (GK) total protein content coincident with a significant decrease (≈48%, $P<0.01$) in GK activity (U/g liver) in the HFD group (2.3±0.4) compared to the CTR group (4.4±0.5). Surprisingly, the decrease in GK protein was not the consequence of attenuation in insulin signal transduction given that phosphorylation of IRS1-Y1222 and Akt-S473 was similar between the HFD and CTR groups.
Conclusion: Four weeks of HFD consumption impaired the effect of hyperinsulinemia and hyperglycemia on NHGU and glycogen synthesis; however, it did not alter the response of either to the portal glucose signal. Although the HFD was associated with a decrease in hepatic GK activity, this was not due to a reduction in the activation of either IRS1 or Akt. These data suggest that alternative control mechanisms are involved in the regulation of hepatic GK, and these may have a significant impact on the ability of the liver to take up glucose.
Supported by: NIH

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Hepatic glycogen supercompensation reduces glycogen synthesis without altering net hepatic glucose uptake in dogs

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Background and aims: A hallmark characteristic of diabetes mellitus is glucose intolerance, which is manifest by postprandial hyperglycemia. Because one-third of an oral glucose load is taken up and metabolized by the liver, efforts are underway by pharmaceutical companies to create medications that would increase liver glucose uptake and glycogen synthesis during the postprandial state. While both hyperinsulinemia and portal vein glucose infusion have been shown to stimulate hepatic glucose uptake, little is known about how an increase in the hepatic glycogen content could affect the responses of the liver to these stimuli. Thus, the purpose of this study was to determine how an acute increase in hepatic glycogen affects the liver's ability to take up and metabolize glucose in response to hyperglycemic/hyperinsulinemia or portal glucose infusion.
Materials and methods: During the first 4h of each study, all dogs received somatostatin and basal amounts of intraportal insulin and glucagon. Blood glucose was doubled by glucose infusion into a peripheral vein and either saline (SAL; n=13) or fructose (5.5 μmol/kg/min; FRU; n=13) was infused intraportally; the latter to trigger liver glycogen loading. The glycogen loading period was followed by a 2h control period, during which basal replacement of hormones was continued but fructose was not infused. A 2h experimental period followed, during which hyperglycemia was maintained. One subset of animals from each group received 4x basal insulin (SAL-INS; n=7 and FRU-INS; n=6) while another received an intraportal glucose infusion (22 μmol/kg/min; SAL-PG; n=6 and FRU-PG; n=7) to assess the effect of the hepatic glycogen content on net hepatic glucose uptake (NHGU) and disposition.
Results: Fructose infusion led to marked differences in hepatic glycogen (345±17 and 538±24 μmol/g liver in SAL and FRU, respectively). The large difference in glycogen had no effect on NHGU (μmol/kg/min) seen in response to hyperinsulinemia (16±4 and 15±4 in SAL-INS and FRU-INS, respectively) or portal vein glucose infusion (19±2 and 17±2 in SAL-PG and FRU-PG, respectively). On the other hand, the percentage of NHGU that was directed to glycogen was reduced ($p<0.04$) by hepatic glycogen supercompensation during hyperinsulinemia (70±3 and 58±10% in SAL-INS and FRU-INS, respectively) and during portal vein glucose infusion (70±6 and 54±3% in SAL-PG and FRU-PG, respectively). Furthermore, these reductions in glycogen synthesis were accounted for primarily by an increase in liver lactate output ($p<0.05$) during both hyperinsulinemia (16±3 and 33±8% in SAL-INS and FRU-INS, respectively) and in response to portal vein glucose infusion (27±6 and 38±4% in SAL-PG and FRU-PG, respectively).
Conclusion: Our data show that acutely increasing the glycogen level from ~350 to ~540 μmol/g had no observable effect upon NHGU, although it caused a reduction in hepatic glycogen synthesis and a compensatory increase in lactate output. Since the reduction in glycogen synthesis seen in response to hyperinsulinemia vs. portal vein glucose infusion were similar, altered activity of glycogenic enzymes are likely responsible for the diminished glycogen synthesis associated with glycogen supercompensation. Thus, as medications are developed to increase postprandial hepatic glucose disposal it should be remembered that although high glycogen levels will not inhibit NHGU, they will increase carbon flux through non-glycogenic pathways.
Supported by: NIDDK

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Per2 plays a major role in the control of liver glycogen metabolism and fasting glycaemia

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Background and aims: Recent evidence suggests that obesity-induced derangements of the expression of molecular components of the circadian clock may be implicated in the development of glucose intolerance. The aim of this study was to investigate the role of the clock protein period 2 (Per2), a key component of the circadian clock whose expression is deranged during obesity, in glucose homeostasis in lean and obese mice.
Materials and methods: To investigate the role of Per2 in glucose metabolism in-vivo we used mice bearing a targeted gene mutation in the Per2 gene (Per2brdm) and thus unable to express a functional Per2 protein. Mice were hosted in our standard mouse facility in a 12-hour light and 12-hour dark cycle. Wt and Per2brdm mice were fed with either standard chow diet or high-fat diet for 24 weeks and analyzed for glucose homeostasis. Glucose tolerance test (GTT) and insulin tolerance test (ITT) were performed on mice food deprived for 7 hours. For pyruvate tolerance test (PTT) mice were fasted for 14 hours. Glucose levels were measured over 12 hours starvation timecourses during the light and the dark phase. Fed and fasting blood glucose and hepatic glycogen content were measured over different circadian timepoints. To investigate the role of Per2 in the control of liver gene expression we performed DNA-microarray analysis of RNA preparations from liver of Per2brdm and WT mice. Results were validated by QPCR.
Results: Our results suggest that Per2 loss of function is not a basic requirement for the development of obesity-induced insulin resistance. Per2brdm mice show similar glucose tolerance compared to WT mice when placed on chow diet, and Per2 loss of function does not predispose to high-fat diet-induced insulin resistance. However, we have identified an important role for Per2 in the control of fasting glycemia and hepatic glycogen metabolism. Per2brdm mice show a decreased fasting glycemia compared to WT controls

during the light phase. This difference is observed after 4 hours of food deprivation, is maximal at 7-8 hours of starvation, and disappears after about 12 hours of starvation. Gene expression-signature analysis from the microarray data indicates decreased expression of genes involved in gluconeogenesis and glycogen metabolism in livers from Per2brdm mice compared to livers from WT mice. PTT was performed to evaluate whole body gluconeogenesis. The results show that WT and Per2brdm mice display similar gluconeogenic potential in a PTT test. To evaluate the role of Per2 in glycogen metabolism we measured hepatic glycogen content in fed or 8 hours fasted WT and Per2brdm mice. The results show that fed Per2brdm mice display lower liver glycogen content during the light phase compared to WT controls. This difference is more pronounced in mice that were starved for 8 hours with Per2brdm mice displaying less than a third of the hepatic glycogen content compared to control WT mice (p-value < 0.005).
Conclusion: Our data suggest that Per2 loss of function is not a major cause or a predisposition factor to impaired glucose tolerance. Nonetheless our results suggest that Per2 plays an important role in the control of fasting glycemia and in hepatic glycogen metabolism.
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Evidence for a key role of hepatic mitochondrial phosphoenolpyruvate carboxykinase in glucose homeostasis *in vivo*
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Background and aims: The cytosolic isoform of phosphoenolpyruvate carboxykinase (PEPCK-C) is generally regarded as the primary enzyme that provides PEP for gluconeogenesis and glyceroneogenesis. The metabolic role of the mitochondrial isoform, PEPCK-M, however, has not been established. In contrast to PEPCK-C whose transcription is strongly inhibited by insulin, we hypothesized that the constitutively expressed PEPCK-M isoform, might instead be regulated in response to fuel supply by mitochondrial GTP- a known sensor of mitochondrial metabolic flux in the pancreatic beta-cells.
Materials and methods: To assess its role, PEPCK-M was silenced using siRNA in primary rat hepatocytes and *in vivo* with antisense oligonucleotides in young, weight-matched Sprague Dawley rats. Gluconeogenesis rates in hepatocytes were assessed using ^{13}C-labeled substrates with analysis by mass spectroscopy. Live awake rats were studied by euglycemic hyperinsulinemic clamps and a 36-hour fast.
Results: An 80% reduction of PEPCK-M mRNA in hepatocytes reduced gluconeogenesis by 60% (P<0.001). Similar reductions in gluconeogenesis with PEPCK-M silencing were observed regardless of the presence of glucose, glucagon, or insulin. PEPCK-M message was silenced 80% in liver and the plasma glucose was significantly reduced in fed rats (160±4 vs. 146±5 mg/dl, P<0.05) but was equivalent following a 36 hour fast (115±2 vs. 116±2 mg/dl, P=0.88). Similarly, fed insulin was reduced (57±6 vs. 35±6, P<0.02) but not with fasting (19±3 vs. 18±6 μU/ml, P=0.87). Euglycemic hyperinsulinemic clamps identified significantly increased insulin sensitivity when PEPCK-M was silenced (GINF: 20±1 vs. 26±1 mg/kg/min, P=0.003) that could not be accounted for by differences in endogenous glucose production nor uptake in the muscle or adipose. Interestingly, post-absorptive hepatic glycogen was 82% lower and plasma triglycerides 42% lower along with a 25% reduction in WAT suggesting a constitutive defect in PEP production.
Conclusion: These data from silenced PEPCK-M both in primary hepatocytes and *in vivo* support an important role for PEPCK-M in glucose homeostasis via gluconeogenesis and possibly glyceroneogenesis. Because of the beneficial effects of lowering glucose, insulin, triglycerides and fat mass with a concomitant increase in insulin sensitivity but without hypoglycemia following a prolonged fast, PEPCK-M may be an attractive target for the treatment of type-2 diabetes.
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Farnesoid X Receptor inhibits glucose-induced LPK gene expression by interfering with Carbohydrate Response Element Binding Protein (ChERBP) activity
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Background and aims: Carbohydrate response element binding protein (ChREBP) is a transcription factor which can be activated in response to glucose to induce the expression of the glycolytic gene Liver Pyruvate Kinase (LPK) as well as some lipogenic genes such as Fatty Acid Synthase (FAS) and Acetyl CoA Carboxylase (ACC1). Previous works in our team have shown that the activation of nuclear receptor FXR (Farnesoid X Receptor) inhibits the glucose-mediated induction of these genes. Our aim is to unravel the molecular mechanisms undergoing the negative effect of FXR on the expression of glycolytic and lipogenic genes.
Materials and methods: Two human hepatocyte cell lines, IHH and HepaRG, were treated with different glucose concentrations and with FXR agonist GW4064. Real-time PCR experiments were performed to analyse the expression of the LPK gene. Western blot analysis was used to study the nuclear localisation of ChREBP. Chromatine immunoprecipitation (ChIP) experiments were performed to follow the binding of ChREBP and FXR to the LPK promoter under different experimental conditions.
Results: We show that FXR activation inhibits the glucose-mediated induction of the LPK gene expression. Our hypothesis is that this inhibition could be due to an interference of FXR with ChREBP transcriptional activity. Our results show that the nuclear receptor FXR interacts physically with the transcription factor ChREBP but this interaction does not interfere with the nuclear translocation of ChREBP or its binding to the LPK promoter. Recent results suggest that the activation of FXR leads to a recruitment of Histone Deacetylases (HDAC) to the LPK promoter since a treatment with Trichostatin A blocks the FXR effect on the LPK gene expression.
Conclusion: These results demonstrate that FXR plays an important role in regulating the glucose-mediated induction of glycolysis and lipogenesis by interfering with ChREBP transcriptional activity.

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Comparison of direct and indirect pathways of hepatic glycogen synthesis using the [1-13C]glucose and deuterated water methods in fed and healthy subjects
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Background and aims: Hepatic glycogen is synthesized by two distinct processes: the classical direct pathway from intact glucose units, and the indirect pathway involving 3-carbon intermediates. It is now well established that the direct pathway is the major route of hepatic glycogen synthesis in healthy subjects but its contribution is significantly reduced in both Type 1 and Type 2 diabetes. Direct and indirect pathway activities can be safely and noninvasively measured using stable isotope tracers to label the glycogen precursors and glucuronidation probes such as Paracetamol to sample the enrichment of UDP-glucose from these tracers. The analysis can be applied in a routine clinical setting and could potentially be a diagnostic tool for assessing hepatic glucose and glycogen metabolism. [1-13C]glucose is the most widely used tracer to quantify the contribution of the direct and the indirect pathways to the glycogen synthesis but it is relatively expensive. Moreover, both plasma and urine need to be collected and analyzed for 13C-enrichment analysis thus incurring a significant analytical burden. Deuterated water (2H2O) has advantages over [1-13C]glucose since it is less expensive and the analysis only requires a single urine sample. To date, the two tracers have not been verified against each other. Therefore, we administered both [1-13C] glucose and 2H2O to healthy subjects and compared the direct/indirect pathway contributions from both tracers for each subject.
Materials and methods: Six overnight-fasted healthy young subjects took a breakfast, were the CHO portion included 10 grams of [1-13C] glucose. A 2H2O load was ingested to attain body water 2H-enrichment of 0.3%. This enrichment was maintained throughout the study by providing drinking water enriched with 0.3% 2H2O. Paracetamol was taken for UDPG sampling as urinary Paracetamol glucuronide and a blood sample was also collected.

Paracetamol glucuronide and blood glucose were derivatized to MAG for 1H, 2H and 13C NMR analysis. Body water 2H-enrichment was also quantified by 2H NMR. Direct pathway from [1-13C] glucose ingestion was calculated by the analysis of excess 13C enrichment of C1 and C6 of MAG obtained from both glucuronide and plasma. Direct pathway from 2H2O was obtained from the 2H5/body water ratio.
Results: Direct pathway measured by 13C and 2H was 59% ± 7.34 and 61% ± 1.88 respectively while indirect pathway was 41% ± 7.34 and 39% ± 1.88. Data are presented as means ± standard errors.
Conclusion: In conclusion, direct and indirect pathway measured by 13C analysis after ingestion of [1-13C] glucose in the postprandial state, matches the data obtained by 2H NMR analysis of 2H enrichment from 2H2O under the same conditions.
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New aspects of hepatic glucose phosphorylation by intracellular glucokinase localisation
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Background and aims: Coupling of millimolar glucose concentrations to liver metabolism is mediated by the glucose phosphorylating enzyme glucokinase (GK) and vital to maintain glucose homeostasis. Only in liver a specific glucokinase regulatory protein (GRP) inhibits GK activity and mediates the GK nuclear import at low glucose. It is poorly understood so far whether GRP affects GK nuclear export at high glucose concentrations. Therefore one aim of this study was to elucidate the role of the GRP in GK nuclear export. Furthermore, a function of GK in the nucleus has not yet been ascertained. Recently we could establish a fluorescence resonance energy transfer (FRET) based glucose specific nanosensor (FLIPglu) to monitor intracellular glucose flux. Thus a second aim of this study was to characterize glucose uptake and metabolism in hepatocytes with respect to the cytoplasmic and nuclear compartments.
Materials and methods: Localization and translocation of GK and GRP were analyzed in primary rat hepatocytes in comparison to MIN6 beta cells by ECFP and EYFP fluorescent fusion proteins and through fluorescence distribution after photoconversion of a Dendra2 or PA-GFP fusion protein, respectively. Dynamic changes in the nuclear and cytoplasmic glucose concentration were determined in perifusion experiments in primary hepatocytes, COS cells and MIN6 cells expressing either the cytoplasmic FLIPglu or the nuclear FLIPglu-nuc.
Results: In hepatocytes a higher portion of the GRP has been identified in the nucleus compared to the cytoplasm. The nuclear/cytoplasmic ratio of the GRP was only marginally affected by glucose. In contrast the nuclear/cytoplasmic ratio of glucokinase was significantly higher at low as compared to high glucose. In MIN6 cells endogenously expressing GK but not GRP, comparable results were observed after overexpression of an EYFP-GRP protein. By selective photoconversion it was demonstrated that one fraction of the GRP protein pool in hepatocytes continuously shuttled between the nucleus and the cytoplasm, both at high and low glucose. While the cytoplasmic fraction of the GRP was completely mobile, in the nucleus an immobile fraction of the GRP was elucidated. Using compartment specific FLIPglu sensor expression it was shown that the cytoplasmic glucose uptake was accompanied by an only slightly time-delayed nuclear glucose uptake irrespective of the analyzed cell type. Interestingly, after removal of extracellular glucose the nuclear glucose concentration decreased twofold faster in hepatocytes compared to MIN6 cells and COS cells, which indicates that glucose phosphorylation takes place only in the nucleus of hepatocytes, where GK is present.
Conclusion: GK leaves the nucleus in a glucose dependent, but GRP independent manner. For the first time we have shown that after dissociation from the GRP, GK is active in the nucleus of hepatocytes. Our results open new therapeutic perspectives for the development of liver specific GK activating compounds interfering with the GK-GRP interactions.

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Insulin resistance alters post-prandial haemodynamic parameters: an impedance cardiography approach
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Background and aims: In postprandial state insulin regulates both metabolic and cardiovascular responses. In insulin resistance (IR) states, insulin action is impaired at both levels. However, the hemodynamic responses of postprandial phase in IR patients are poorly characterized. Impedance Cardiography (ICG), a relatively easy-to-use technique, allows a reliable beat-to-beat non invasive haemodynamic evaluation. We investigated cardiac/vascular hemodynamic response by ICG technique in fasting and postprandial state, in subjects with and without IR.
Materials and methods: In 66 volunteers (50 male, 16 female, age 48±8 years), without subclinical atherosclerosis, a mixed meal (10 kcal/kg; 55% carbohydrate, 15% proteins, 5% fats) was administered while basal and post-meal (180 min) continuous ICG monitoring was performed. Insulin sensitivity (Si) was determined by the minimal model analysis, and the following haemodynamic parameters were recorded: systolic and diastolic arterial blood pressure (BP), heart rate (HR), stroke volume (SV), cardiac index (CI), systemic vascular resistance index (SVRI), left cardiac work index (LCWI).
Results: According to the median value of Si (7.27×10^{-4}dl•kg^{-1}•min^{-1} per μU/ml) subjects were divided in insulin-resistant (IR; n 33) and insulin sensitive (IS; n 33). In fasting condition, IR showed higher values of systolic (p=0.003) and diastolic (p=0.002) BP and SVRI (p=0.011), compared to IS. In post-prandial state vasodilatation was comparable and synchronous (at 30 min) in IR versus IS (SVRI respectively -12.7±2.5% and -3.6±2.5%; p M-ANOVA 0.209), but the subsequent recovery (30-180min) was significantly impaired in IR (M-ANOVA 0.018). In post-prandial state haemodynamic parameters linked to cardiac function were not statistically different in IR vs IS.
Conclusion: IR subjects show a worse cardiovascular performance compared to IS in fasting condition. In post-prandial phase, IR associates with a shorter duration of vasodilatation in the absence of an altered cardiac performance. Hemodynamic alterations in both fasting and postprandial states are important features of insulin resistance.

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Assessment of insulin signalling in isolated circulating leukocytes is feasible and parallels whole-body insulin resistance in prednisolone-treated healthy men
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Background and aims: Impaired insulin-stimulated glucose disposal in skeletal muscle is a hallmark of type 2 diabetes mellitus (T2DM). Thus, several defects in the insulin-signalling cascade in skeletal muscle were reported in subjects with impaired glucose metabolism or T2DM. Assessment of these defects in vivo requires laborious and invasive skeletal muscle biopsies. The aim of this study was to investigate whether it would be feasible to measure insulin signalling in more easily accessible circulating leukocytes. To this end, healthy men were treated with low- or high dose prednisolone in order to induce various levels of insulin resistance.
Materials and methods: Healthy men (n=21; mean±SE: age: 21±2; BMI: 22±2) underwent a hyperinsulinaemic-euglycaemic clamp at baseline and on day 14 of treatment with either prednisolone 30 mg daily (PRED30), prednisolone 7,5 mg daily (PRED7.5) or placebo (PLB). Blood samples were obtained at t=-5 min, t=5 min, t=10 min and t=20 min during insulin infusion (at 40 mU/m2•min) for isolation of peripheral leukocytes using an erythrocyte lysis buffer. The expression and phosphorylation (at Thr246) of

proline-rich Akt substrate of 40 kDa (PRAS40), a marker of the insulin-signalling cascade, was assessed by immunoblotting and densitometry analysis. Insulin sensitivity was quantified as the M-value, obtained during 90-120 min of insulin infusion.
Results: Both PRED7.5 and PRED30 vs. PLB decreased insulin sensitivity (mean difference -2.8±1.0 mg/kg•min, P = 0.03 and -4.5±0.9 mg/kg•min, P = 0.001 respectively). Insulin infusion increased phosphorylated PRAS40 (P-PRAS40)/total PRAS40 ratio at t=5 min, t=10 min and t =20 min as compared to t=-5 min by 35% (P < 0.05 for all). During treatment, P-PRAS40/total PRAS ratio was decreased in the PRED30 group (P = 0.05), but not in the PRED7.5 arm. The change in P-PRAS40/total PRAS40 ratio during treatment correlated with the change in M-value (R=0.447, P = 0.042).
Conclusion: Molecular aspects of insulin signalling and changes thereof can be measured in peripheral leukocytes and correlate with whole-body insulin sensitivity. Although the physiological role of these assessments in leukocytes requires further study, this method may provide an alternative, less-invasive tool to monitor changes in insulin signalling in health and disease.

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Meal-related increases in microvascular vasomotion are impaired in obese individuals
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Background and aims: Steady state hyperinsulinaemia during a hyperinsulinaemic euglycaemic clamp stimulates endothelium-dependent vasomotion as well as capillary recruitment, which contribute to increased glucose uptake; these phenomena have been shown to be blunted in obesity. If insulin's effects on microcirculatory function indeed play a physiological role in regulating insulin-mediated glucose uptake, such effects should be demonstrable not only during steady-state hyperinsulinaemia, but also after meal ingestion. The aim of the present study was to investigate the effects of an oral glucose load and a liquid mixed meal on cutaneous microvascular vasomotion in lean and obese subjects.
Materials and methods: A randomised, placebo-controlled trial was performed in 18 lean (BMI 22.5±1.7 kg/m^2) and 13 obese (BMI 34.0±3.5 kg/m^2) subjects, to examine the effects of a glucose drink (75 g glucose), a 495-kcal liquid mixed meal (60% carbohydrates, 25% proteins, 15% fat) or placebo (tap water) on microvascular function. Skin blood flow was measured by laser Doppler flowmetry (LDF). Vasomotion was examined by Fourier analysis of the LDF signal.
Results: Both the glucose drink and the liquid mixed meal, but not the water drink, induced hyperinsulinaemia. The levels of hyperinsulinaemia were higher in obese compared to healthy subjects (glucose drink: 98.1±82.5 vs. 39.2±17.0 mU/l, P<0.05; mixed meal: 99.8±78.9 vs. 52.7±20.5 mU/l P=0.07). Water intake did not alter the energy density of any of the five frequency components in either the lean and obese group. Intake of the glucose drink increased the endothelial component of vasomotion (P<0.05) in lean subjects. In lean subjects, intake of the mixed meal drink increased the energy density of all five frequency components (P<0.01, P<0.01, P<0.01, P<0.01, and P<0.05, respectively), and the total energy density of the entire spectrum (P<0.01). In obese individuals, neither the glucose nor the mixed meal drink had any effect on the energy density of the five frequency bands or the total energy density. The increase in the energy density of the endothelial, neurogenic, and respiratory frequency components and the total energy density in lean individuals in response to the mixed meal drink was significantly different from the response to the mixed meal drink in obese individuals (P<0.01, P<0.05, P<0.05, and P<0.01, respectively).
Conclusion: Our data demonstrate that ingestion of a meal increases microvascular vasomotion in lean individuals. In addition, we found that the increase in microvascular vasomotion with meal ingestion was impaired in obese individuals and that this lack of meal-induced stimulation of microvascular vasomotion paralleled blunted insulin-stimulated glucose disposal after meal feeding in these subjects. Thus, these data are consistent with a physiological role for insulin-stimulated microvascular vasomotion in insulin-mediated glucose uptake in the postprandial state.

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Effects of starvation on insulin sensitivity and glucose metabolism in obese patients with type 1 diabetes mellitus
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Background and aims: At present time obesity is more frequently connected with type 1 diabetes mellitus (T1DM) in western population. The effect of reduced energy intake and several days of fasting is not fully known in this group of diabetic patients. The aim of our work was to study the influence of seven days of starvation on insulin sensitivity and glucose metabolism in obese patients with T1DM.
Materials and methods: We studied 14 obese patients with T1DM (42,6±9,4 years, BMI 32,4±2,1 kg m^{-2}) and 13 non-obese control patients with T1DM (36,9±13,9 years, BMI 22,6±2,1 kg m^{-2}). The insulin sensitivity and glucose oxidation were measured in obese T1DM patients before starvation, immediately after 7 days of starvation and 21 days thereafter. Control group was studied only after overnight starvation. Insulin sensitivity was measured using hyperinsulinemic euglycemic clamp (the 2-step hyperinsulinemic euglycemic clamp lasting 6 hours, period 1: 0 to 120 minutes 1 IU/kg/min of insulin and period 2: 120 to 360 minutes 10 mU/kg/min of insulin - Humulin R, Elly-Lilly, USA). Glucose oxidation and non-oxidative glucose disposal were measured before and during the clamp by indirect calorimetry - ventilated canopy system (VMAX, Sensormedics, Anaheim, USA). Evaluations of urinary urea nitrogen excretion was made to calculate protein oxidation. Mean±SD, T-test, ANOVA were used for statistical evaluation.
Results: All patients tolerated the period of starvation. Obese T1DM patients lost 6,1±1,1 kg. Glycaemia during starvation was maintained at 5 mmol/l by adjustment of basal insulin dose. Starvation reduced insulin-mediated glucose disposal in both phases of clamp (P<0,001). This was caused mainly by reduced glucose oxidation after starvation period (P<0,001). Non-oxidative glucose disposal was not changed.
Conclusion: One week of starvation transiently decreased insulin mediated glucose disposal in obese T1DM patients. This was namely caused by reduced glucose oxidation. To our best knowledge we are the first who described this effect.

Insulin infusion rate 10mU/kg/min,*P<0,001 obese during the time, #P<0,05 obese vs controls

Obese T1DM (n=14)	Before starvation	After starvation	21 days after starvation	Controls (n=13)
Glucose disposal (mg/min/kg)	9,69±1,48#	6,78±1,21*	9,31±1,16	12,02±2,16
Glucose oxidation (mg/min/kg)	2,81±0,52	0,88±0,98*	2,80±0,67	3,54±1,17
Non-oxidative glucose disposal (mg/min/kg)	6,88±1,44#	5,94±0,87	6,51±1,03	8,48±1,58

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Adipocyte fatty acid binding protein in diabetes mellitus - effects of hyperinsulinaemia and acute angiotensin II type 1 receptor blockade
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Background and aims: Beside of its regulatory role in lipid metabolism, adipocyte fatty acid binding protein (A-FABP) has been suggested to be involved in the development of insulin resistance. We investigated: a) plasma concentrations of A-FABP in parallel with its expressions in subcutaneous adipose tissue (SAT) and b) their response to acute hyperinsulinaemia and acute angiotensin II type 1 receptor blockade (ARB) in type 2 diabetes.
Materials and methods: 11 patients with type 2 diabetes (D) and 12 healthy age-matched control subjects (C) underwent: 1) hyperinsulinaemic-euglycaemic clamp (HEC); 2) HEC after acute ARB (losartan 200 mg) (AT-HEC) and 3) saline infusion (SAL) as a volume control examination. At 0 min and

240 min of the interventions blood sampling for assessment of plasma A-FABP and needle biopsies of abdominal SAT were performed. Adipose tissue samples were processed for real-time PCR method to quantify gene expressions of A-FABP, E-FABP (epidermal FABP, a minor FABP-isoform of adipose tissue) and PPAR-γ. For statistical analysis ANOVA with repeated measures was used (significances in Table: [a] Treatment effect p<0.05 - difference between interventions: HEC vs. AT-HEC vs. SAL; [b] Time effect p<0.05 - changes between time points: 0 min vs. 240 min).

Results: Plasma A-FABP was 1.6-fold higher in D relative to C (p<0.001). In D, a comparable decrease in plasma A-FABP was detected during both clamps, whereas no changes were observed during SAL. In C, time profiles of A-FABP differed between the clamps, showing an increase in basal concentrations in AT-HEC. A-FABP expression in SAT was 3.0-fold higher in D (p<0.001) without any dynamic changes during all interventions. Plasma A-FABP correlated positively with its expression (r=+0.59; p<0.001). Both A-FABP plasma concentrations and mRNA expressions were independently associated with BMI, waist circumference, glycaemia, insulinaemia and glucose disposal. E-FABP showed higher expressions in C (p<0.001). A-FABP/E-FABP mRNA ratio was 3.0-fold higher in D compared to C (p<0.001) without any changes within and between clamps. PPAR-γ expressions were lower in D compared to C (p<0.01), no dynamic changes were shown.

Conclusion: A-FABP plasma concentrations as well as expressions are increased in type 2 diabetes and they are closely associated with parameters of obesity, insulin resistance and hyperglycaemia. On the contrary, the expressions of E-FABP and PPAR-γ are decreased in diabetes. Hyperinsulinaemia differentially regulates A-FABP in D compared to C. In C but not in D, losartan stimulates basal A-FABP plasma concentrations without any effect on its expressions.

A-FABP plasma concentrations and expressions of selected genes in SAT. Data shown as mean (95% CI).

Variable		D (n=11)		C (n=12)		Group effect (p value)
		0 min	240 min	0 min	240 min	
A-FABP plasma concentrations (ng/ml)	HEC	21.31 (19.3-23.45)	18.85[b] (17.15-20.74)	12.56[a] (11.3-14.0)	12.64 (11.38-14.09)	<0.001
	AT-HEC	23.4 (21.2-25.77)	20.02[b] (18.08-22.18)	15.24[a] (13.64-17.08)	12.73[b] (11.45-14.19)	
	SAL	22.42 (20.23-24.86)	21.64 (19.53-23.99)	13.86[a] (12.37-15.59)	15.20[b] (13.61-17.03)	
A-FABP mRNA / Cyclophilin mRNA	HEC	1937 (1686-2314)	1971 (1695-2401)	718.4 (647.6-808.6)	650.3 (592.8-721.8)	<0.001
	AT-HEC	2004 (1732-2422)	1940 (1688-2318)	758.3 (679.2-860.9)	675.6 (610.0-758.9	
E-FABP mRNA / Cyclophilin mRNA	HEC	196.5 (181.0-214.3)	184.1 (170.2-200.0)	257.8 [a] (210.7-322.7)	238.5 (196.3-295.9)	<0.001
	AT-HEC	198.1 (182.4-216.1)	184.7 (169.8-201.8)	292.9 [a] (232.5-380.1)	259.8 (212.2-325.6)	
PPAR-γ mRNA / Cyclophilin mRNA	HEC	139.6 (112.4-166.3)	158.9 (132.1-185.3)	169.8 (116.3-243.5)	168.1 (115.1-241.2)	<0.01
	AT-HEC	137.0 (109.8-163.8)	158.9 (132.1-185.2)	167.5 (111.1-247.3)	202.5 (140.0-288.2)	

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Fasting and postprandial hepatic glycogen content is not altered by systemic insulin delivery in type 1 diabetic patients after successful pancreas kidney transplantation

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Background and aims: Insulin replacement in Type 1 diabetes mellitus (T1DM) is usually performed via a systemic (subcutaneous) instead of the physiologic portal route. So far it is unclear whether this systemic route of insulin delivery might contribute to metabolic defects in these patients. Successful pancreas kidney transplantation (PKT) with systemic venous drainage is an ideal model of optimized systemic insulin therapy. Therefore, the aim of the present study was to investigate the effects of PKT on fasting and postprandial liver glycogen content.

Materials and methods: Using 13C-nuclear magnetic resonance spectroscopy (13C-NMR), liver glycogen concentrations were assessed in 9 T1DM patients after successful PKT (24±1kg/m², 47±3yrs, 3f/6m, fasting glucose 84±3mg/dl, HbA1c 5.1±0.2%) with systemic venous drainage and in 9 matching nondiabetic controls (CON) (24±1 kg/m², 47±3yrs, 3f/6m, 89±3 mg/dl, 5.4±0.1%) at fasting and after two standardized mixed meals.

Results: Liver glycogen concentrations at fasting (PKT:195±12, CON:209±10mM), after breakfast (PKT:216±12, CON:233±11mM) and after lunch (PKT:230±13 vs. CON:171±15mM), as well as the increment of liver glycogen content after the meals were comparable in PKT and CON. Mean and fasting concentrations of glucose, insulin, C-peptide and glucagon were similar in PKT vs. CON.

Conclusion: Despite systemic insulin secretion, T1DM after successful PKT exhibit unchanged fasting and postprandial liver glycogen stores in comparison with age-and BMI-matched non-diabetic controls. Thus, this study indicates that systemic insulin substitution does not cause alterations of hepatic glycogen storage.

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The effect of liraglutide on metabolism in ApoE-/- mice with RNAi-mediated adiponectin gene inhibition

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Background and aims: Liraglutide is an analogue of Glucagon-like peptide-1(GLP-1), researched and developed by Novonordisk. It can bind with the GLP-1 specific receptor, which belongs to the Islets of Langerhans β cells, promote the expression and biosynthesis of the pre-insulin gene, and facilitate the secretion of insulin in the genetic level. But its specific mechanism retains unknown. In our study, we have investigated the effects of Liraglutide on insulin sensitivity and glucose-lipid metabolism in $ApoE^{-/-}$ mice with RNAi-mediated adiponectin gene inhibition.
Materials and methods: High-fat diet fed male apoE-/- mice were randomly divided into adiponectin shRNA adenovirus injection group (ADI group, n=8), Liraglutide co-injection of adiponectin shRNA adenovirus group (HEA group, n=8) and adenovirus control group (GF group, n=6). The hyperinsulinemic-euglycemic clamp combined with 3-[3H] glucose was used as a tracer to assess the insulin sensitivity. Plasma FFA, insulin concentrations (PIns), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), TG and TC concentrations were measured.
Results: In the IVGTT, the group with 1mg· kg^{-1} Liraglutide, Bid, at time points of 5, 15, 30 min after glucose challenge, presented lower significantly than other groups in the blood glucose(P<0.01), with a significant increase in the plasma insulin at the points of 5 and 15 min(P<0.01). Fasting blood glucose (FBG), body weight, Free fatty acids (FFA),TC,TG,LDL-C,HDL-C and fasting plasma insulins (PIns) in $ApoE^{-/-}$ mice with Liraglutide and adiponectin shRNA adenovirus (HEA group) co-injection of were significantly lower than those with high-fat diet and adiponectin shRNA adenovirus injection (ADI group) (P<0.01). However, HDL-C showed a significant elevation, compared HEA with ADI group (P<0.05). During the steady-state of clamp, plasma insulin in ADI group was significantly higher than that in HEA group (P<0.01). Although FFA, TC and TG were suppressed in all groups, they were still higher in ADI group than those in HEA group(P<0.05). Glucose infusion rate(GIR) in HEA group were significantly higher than ADI group (P<0.01). In the end of clamp, glucose disappearance rate (GRd) was significantly lower, and HGP significantly higher in ADI group than HEA group (P<0.01).
Conclusion: Liraglutide administration can improve insulin resistance by increasing plasma adiponectin level in ApoE-/- mice with RNAi-mediated adiponectin gene inhibition.
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Normalising effect of GLP-1 and Exendin-4 on the deleterious hepatic metabolism, of an obesity model

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Background and aims: Obesity is often related with several metabolic alterations that can drive to insulin resistance and finally diabetes. GLP-1 and its structurally homologous peptide, exendin-4 (Ex-4), both have insulinotropic and antidiabetic actions and stimulatory effects on the glucose metabolism of extrapancreatics tissues such as liver and muscle. Here, we have studied the effect of GLP-1 and Ex-4 treatment, on Glut-2 expression and other parameters, in an obesity model (Ob) compared to normal (N).
Materials and methods: Ob model was obtained in adult Wistar rats by chronic feeding -5 weeks- with a "cafeteria diet", consisting in standard chow supplemented with cookies, liver paste, bacon, and whole-milk containing sucrose (333 g/l) and 10 g/l of a mineral and vitamin complex (65% energy derived from lipids). The N group was fed with standard chow and water *ad libitum* (8% calories from fat). Although weight was not different between N and Ob, the Ob model showed fasting plasma glucose, triglycerides (159±14 mg/dl, n=9) and cholesterol (94±4 mg/l, n=10) higher than N (overall mean: 145±7% Δ N-rats, p<0.001); no significant differences with N were detected in plasma insulin or GLP-1 -RIA-. Ob (n=5-10 rats/group) and N (4-10 rats/group) were treated (3 days), through an osmotic pump, with saline (control), GLP-1 (0.86 nmol/kg/h) or Ex-4 (0.1nmol/kg/h). Blood samples were taken before and by the end of treatment for plasma measurements. In liver, we analyzed Glut-2 expression -protein, by Western blot, and mRNA, by RT-PCR- and also glycogen content -antrona method-.
Results: In Ob, liver Glut-2-mRNA was much lower than that in N animals (0.41±0.02 times N-control, n=10 rats, p<0.001), while Glut-2-protein was higher (123±10% N-control, n=14, p<0.05); GLP-1 treatment reduced the Glut-2-protein levels (64±4% Ob-control, n=7, p<0.001), without altering the mRNA value (0.96±0.04 times Ob-control, n=5); treatment with Ex-4 lowered the Glut-2-protein (60±4% Ob-control, n=7, p<0.001) to a value close to that in N (84±7% N-control), without modifying the mRNA expression (1.02±0.06 times Ob-control, n=5). The liver glycogen content in Ob rats (902±52 μg/mg protein, n=6), compared to than that in N animals (395±34 μg/mg protein, n=6), was much higher (228±13% N-control, n=6, p<0.001); treatment with GLP-1 reduced the liver glycogen content in Ob, to a value (396±15 μg/mg protein, n=5, p<0.001) undistinguible from that in N rats; Ex-4 also exerted a lowering effect on glycogen content in Ob rats (81±5% Δ Ob-control, n=5, p<0,02), to a level though (726±45 μg/mg protein, n=5, p<0.05) still higher (p<0.001) than that shown in the N group. Plasma glucose and insulin were not apparently different in any group or condition; yet, in the Ob group, GLP-1 lowered the higher than N triglycerides (-50±4% Δ Ob-basal, p<0.04, n=5 rats) to a value even lower than that in N (76±7% N-control, p<0.02), without modifying that of cholesterol; Ex-4 induced a reduction in both parameters, the magnitude of its effect on cholesterol being small but clear (-12±3% Δ Ob-basal, p<0.01, n=6), and that on triglycerides (-57±6% Δ Ob-basal, p<0.01, n=5) as that exerted by GLP-1.
Conclusion: Both, GLP-1 and Ex-4 have a normalizing effect on the high hepatic glycogen accumulation in this obesity model, perhaps through their lowering action upon the higher than normal liver Glut-2 expression, mainly at the translational process, and by reducing the also higher than normal circulating triglycerides levels.
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Enhanced GIP and attenuated GLP-1 incretin effects during IVGTT in Wistar rats under DPP-4 inhibition

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Background and aims: DPP-4 (dipeptidyl peptidase-4) inhibition has been reported to increase incretin effects of GIP as well as GLP-1 by prolonging their half life. DPP-4 cleaves GIP into dipeptide and the GIP metabolite GIP (3-42), whereas the GLP-1 (7-36) amide is cleaved to GLP-1 (9-36) amide. Based on studies in man, pig and rat, these peptides are involved in various glucoregulatory processes. We have recently developed a test to differentiate the insulinotropic effects of both peptides in rats. In the present study, we compared the incretin effects of the intact GIP and the GLP-1 (7-36) amide during IVGTT following DPP4 inhibitor administration.
Materials and methods: Groups of catheterized Wistar rats were tested: placebo (P; 0.1 % BSA), DPP4 inhibitor (INH; 30 μmol/kg P32/98, p.o.), incretin in the absence (+P) and presence of INH (+INH) three days apart in a random order. 4.0 nmol/kg GLP-1 ((7-36) amide; Neo MPS; Strasbourg, France) or 2.0 nmol/kg GIP (Probiodrug AG, Halle/Saale, Germany) were injected. Samples for blood glucose (BG) and insulin (I) were taken (-5, 0 min and at 1, 2, 3, 5, 7, 10, 15, 25, 40 and 60 min). INH was given at -20 min, the incretin at -5 min and the IVGTT (0.4 g glucose/kg) commenced at 0 min. G- and I- $AUC_{0\text{-}25min}$, insulinogenic Index iI (I- and G-AUC area quotient (ng/mmol) was selected to measure incretin effects), glucose efflux K_G (min^{-1}; Conard (1959)) was calculated from IVGTT curves. Groups were compared with two-tailed t-test with Bonferroni-Holm correction, a *p<0.05 was considered significant.
Results: INH significantly increased the effects of GIP - G-AUC (P+P: 220±34, INH+P: 185±17, P+GIP: 157±14*, INH+GIP: 156±25* mmol·min/L), I-AUC (P+P: 77±33, INH+P: 60±19, P+GIP: 115±34, INH+GIP: 153±39* ng·min/mL) and Ii (P+P: 0.35±0.17, INH+P: 0.33±0.11, P+GIP: 0.74±0.24*, INH+GIP: 0.99±0.26* ng/mmol) and improved glucose efflux K_G (P+P: 14.5±4.1, INH+P: 14.2±2.4, P+GIP: 19.8±3.1*, INH+GIP: 20.5±5.0* min^{-1}). In contrast, INH attenuated GLP-1 effects - G-AUC (P+P: 226±33, INH+P: 182±20*, P+GLP-1: 183±24, INH+GLP-1: 215±14 mmol·min/L), I-AUC (P+P: 63±28, INH+P: 65±33, P+GLP-1: 125±19*, INH+GLP-1: 106±38 ng·min/mL) and Ii (P+P: 0.27±0.10 , INH+P: 0.35±0.16 , P+GLP-1: 0.70±0.18*, INH+GLP-1: 0.50±0.19

ng/mmol) and declined glucose efflux K_G (P+P: 12.6±5.1, INH+P: 14.5±3.0, P+GLP-1: 14.9±3.1, INH+GLP-1: 11.1±3.7 min^{-1}).
Conclusion: DPP4 inhibition revealed that GLP-1 and GIP differ remarkably in their glucoregulatory actions in healthy rats. These previously unrecognized actions of DPP4 inhibitors may have implications for the use of DPP-4 inhibitors in humans.
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GLP-1 and somatostatin are part of a paracrine feedback loop in the isolated perfused porcine ileum
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Background and aims: When DPP-4 inhibitors are administrated, peripheral plasma concentrations of intact glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) increase; however, overall (total) incretin secretion appears to be inhibited. The mechanism is unknown, but it has been suggested that increased concentrations of intact GLP-1 may feedback on neighbouring somatostatin-releasing D-cells, which in turn increase their tonic, paracrine restraint on L-cell secretion.
Materials and methods: We examined whether somatostatin (SS) is involved in the local regulation of GLP-1 secretion using the isolated perfused porcine ileum (n=8). In an attempt to break the paracrine circuitry, we infused the GLP-1 receptor antagonist, exendin 9-39 (Ex 9-39)(0.1umol/l), a somatostatin receptor subtype 5 (SSTR5) antagonist (0.1umol/l), or GLP-1(7-36)(1nmol/l), either alone, or in combination with SSTR5 antagonist. Glucagon-like peptide-2 (GLP-2) is co-secreted with GLP-1 from the L-cells, and was therefore used as an index of L-cell secretion.
Results: GLP-1 infusion significantly increased SS secretion from a prestimulatory level of 3.68 ± 0.67 to stimulatory 6.70 ± 1.46 fmol/min (p=0.0195). Moreover, infusion of GLP-1 together with SSTR5 antagonist blocked the inhibitory effect of SS on endogenous L-cell secretion (GLP-2 output increased to 134.7 ± 11.6 % of basal; p=0.0391), resulting in augmented SS output from a prestimulatory level of 3.81 ± 0.64 to stimulatory 9.11 ± 2.34 fmol/min (p=0.0039). Infusion of either SSTR5 antagonist or Ex 9-39 alone resulted in a significant increases L-cell secretion (total GLP-2 output; p=0.0234 and p=0.0391, respectively).
Conclusion: These results indicate that SS regulates the L-cells via SSTR5, and that there is a negative feedback loop between GLP-1 and SS. Additionally we found that GLP-2 secretion was increased by Ex 9-39 alone, indicating that GLP-1 has an indirect tonic inhibitory effect on the L-cells. This feedback mechanism may explain the decreased secretion from the L-cell during DPP-4 inhibitor, even though the concentration of intact GLP-1 plasma concentration increases.

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Glucose-dependent insulinotropic polypeptide regulates cytokine expression and lipolysis in human adipocytes
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Background and aims: Serum levels of IL-6, IL-1ß and IL-1 receptor antagonist (IL-1Ra) as well as of glucose-dependent insulinotropic polypeptide (GIP) are increased in obese individuals. The so-called low-inflammatory state in obesity is associated with adipose tissue dysfunction, leading to altered secretion of cytokines, lipolysis and insulin resistance. Inhibition of GIP signalling in rodents protected against diet-induced obesity and insulin resistance. Since GIP is pro-lipolytic in adipocyte-cultures, we hypothesized, that GIP might also be involved in the induction of an inflammatory state in human adipocytes.
Materials and methods: Using a human in vitro model of preadipocyte-derived adipocytes we analyzed the effect of GIP treatment on mRNA expression of IL-6, IL-1ß and IL-1Ra. Human adipocytes were exposed to recombinant human GIP(1-42), the beta subunit of IkappaB kinase (IKK-ß) inhibitor sc-514 and to recombinant human IL-1Ra (rhIL-1Ra). mRNA expression was analyzed by real-time PCR. Glycerol content in the cell culture supernatant was determined as an index of lipolysis.
Results: Treatment of differentiated human adipocytes with GIP from 10pM to 100nM for 1h induced IL-6, IL-1ß and IL-1Ra mRNA expression. Treatment with 1nM GIP maximally increased IL-6 to 4.21±0.28 fold (p<0.001 vs. control) and IL-1ß to 1.64±0.13 fold (p<0.05 vs. control). IL-1Ra mRNA expression was induced to a maximum of 23.5±3.94 fold (p<0.001 vs. control) with 100nM GIP. During time-course experiments with 1nM GIP, IL-6 mRNA expression was acutely increased to 2.55±0.5 fold (p<0.001 vs. control) after 1h. IL-1ß gene expression was constantly increased during 24h with a maximum expression of 1.64±0.13 fold (p<0.001 vs. control) after 4h of 1nM GIP treatment. IL-1RA mRNA expression was upregulated by 4.77±0.5 fold (p<0.001 vs. control) after 2h and reached a maximal increase by 5.2±0.53 fold (p<0.001 vs. control) at 4h. Preincubation with sc-514 (100µM) for 1h inhibited GIP-induced IL-6 expression by 57% (p<0.05 vs. GIP 1nM 1h) and GIP induced IL-1Ra expression by 78% (p<0.01 vs. GIP 1nM 1h), while GIP-induced IL-1ß expression was not inhibited. Preincubation with rhIL-1RA 1µg/ml inhibited GIP-induced IL-6 expression by 68% (p<0.01 vs. GIP 1nM 1h). Treatment with 1nM GIP for 6h in serum free conditions revealed a 1.64±1.37 fold (p<0.01 vs. control) increase of basal glycerol release. Pretreatment with sc-514 (100µM) fully blocked GIP-induced glycerol release (p<0.001 vs. GIP 1nM 6h) while 1µg/ml rhIL-1Ra 1h prior to 1nM GIP reduced GIP-induced lipolysis by 66% (p<0.05 vs. GIP 1nM 6h).
Conclusion: Our findings demonstrate the potential of GIP to induce a pro-inflammatory state with a concomitant anti-inflammatory IL-1Ra response in human adipocytes, leading to lipolysis. The GIP effect on IL-6 and IL-1Ra mRNA expression and lipolysis seems to involve the classical NF-kB pathway and suggests the involvement of an autokrine IL-1ß effect.

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Effects of glucagon like Peptide-1 and metformin on the proliferation, apoptosis and function of glucagon like Peptide-1-secreting cells
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Background and aims: Glucagon-like peptide-1 (GLP-1) is an incretin hormone, secreted from gut L-cells upon nutrient intake, and forms the basis for novel drugs against type 2 diabetes. Secretion of GLP-1 is impaired in type 2 diabetes. Little is known about the influence of antidiabetic drugs on L-cell function and regeneration. The aim of this study was to investigate the effects of GLP-1 and metformin on the proliferation, apoptosis and function of the GLP-1-secreting cells and the mechanisms underlying such effects.
Materials and methods: The GLP-1-secreting cell line GLUTag was cultured in DMEM media. Cell proliferation was evaluated by ^{3}H-thymidine incorporation, MTT-assay and Ki67 staining after a 48 h-incubation with the antidiabetic agents in the presence or absence of the GLP-1 receptor antagonist exendin(9-39). Cellular cAMP production and secretion of GLP-1 from the cells was measured using specific ELISA kits. Apoptosis was evaluated using cell death detection ELISA and caspase-3 activity was measured using caspase-3 activity ELISA kit. The expression of the GLP-1 receptor in GLUTag cells was assessed by Western blotting using anti-GLP-1 receptor antibody.
Results: GLP-1 stimulated proliferation of the GLP-1-secreting GLUTag cells to a similar extent as insulin at low (3 mM), but not high (11 mM), concentrations of glucose. The DPP-IV inhibitor sitagliptin also promoted proliferation of GLUTag cells at high glucose. These cells expressed the GLP-1 receptor as demonstrated by Western blotting. The stimulatory effect of GLP-1 on GLUTag cell proliferation was blocked by pre-treatment with the receptor antagonist Exendin(9-39). However, the cAMP agonist Sp-cAMP[S] suppressed cell proliferation under these conditions. Metformin significantly inhibited proliferation of GLUTag cells but dose-dependently protected the cells from palmitate-induced apoptosis. In contrast, GLP-1 did not influence palmitate-induced apoptosis of the GLUTag cells. In addition, metformin significantly stimulated short-term secretion of GLP-1 from these cells.
Conclusion: The present study shows that GLP-1 stimulated proliferation of GLP-1-secreting cells, an effect that was blocked by a GLP-1 receptor antagonist, indicating a requirement of the GLP-1 receptor for this event. The results suggest that a positive feedback mechanism by GLP-1 may stimulate these cells in an autocrine/paracrine manner. Since serum GLP-1 levels are decreased in type 2 diabetic patients, the mitogenic stimulation of GLP-1-producing cells by GLP-1 and the protective effect of metformin against lipoapoptosis noted here suggests a novel beneficial long term effect of these antidiabetic drugs in clinical practice, *i.e.* by restoring GLP-1 deficiency through enhanced L-cell mass.
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GLP-1 inhibits glucagon secretion from human alpha cells by a direct effect

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Background and aims: Glucagon is secreted from the alpha-cells of the pancreatic islets. Both intrinsic and paracrine mechanisms are involved in the metabolic regulation of glucagon release. Glucagon secretion is also under hormonal control, one of which is glucagon-like peptide 1 (GLP-1). GLP-1 enhances insulin release but most importantly its hypoglycaemic action is amplified by a concomitant suppression of glucagon secretion. Oversecretion of glucagon occurs in type-2 diabetes, exacerbating the hyperglycaemia resulting from the lack of insulin and this constitutes a valuable feature of GLP-1 action. However, the mechanism by which GLP-1 modulates islet glucagon secretion remains unclear. Here we have examined the effects of GLP-1 on glucagon secretion in human pancreatic islets.
Materials and methods: Immunocytochemistry was used to examine expression of GLP-1 receptors in human islet cells. Islet hormone release was measured from intact islets by static incubation. Confocal microscopy was used to investigate the effects of GLP-1 on the spontaneous $[Ca^{2+}]_i$ oscillations at 1 mM glucose to reflect the activity of alpha-cells. Perforated patch whole-cell measurements were used for membrane potential recordings.
Results: Human pancreatic beta- and delta-cells exhibited clear GLP-1 receptor (GLP-1R) immunoreactivity but only ~1% of the alpha-cells showed detectable levels of the receptor. GLP-1 (10 nM) inhibited glucagon secretion elicited by 1 mM glucose (1G; mean±sem as pg/islet/h: 1G: 20.27±4.06; 1G+GLP-1: 10.83±1.96; $p<0.05$). This effect was not associated with any stimulation of insulin secretion (as ng/islet/h; 1G: 0.15±0.02; 1G+GLP-1: 0.18±0.03) but correlated with a ~3-fold stimulation of somatostatin (SST) secretion (as fmol/islet/h: 1G: 0.35±0.08; 1G+GLP-1: 0.63±0.1; $p<0.05$). The inhibitory action of GLP-1 on glucagon secretion was only partially antagonised by the SST receptor antagonist CYN154806 (100 nM) and GLP-1 retained a 35% inhibitory effect (1G: 3.44±0.41; 1G+GLP-1: 1.58±0.15; $p<0.001$; 1G+CYN154806: 3.74±0.76; 1G+CYN154806+GLP-1: 2.36±0.25; $p<0.05$). Effects of GLP-1 and glucose were next compared with forskolin (10 nM) which blocked glucagon secretion by 30% (1G: 15.76±2.99; 1G+forskolin: 11.06±2.16; $p<0.05$). GLP-1 inhibition was mimicked by the P/Q-type Ca^{2+}-channel blocker ω-agatoxin (AGA; 100 nM; 1G: 10.41±1.7; 1G+GLP-1: 3.85±0.86; 1G+AGA: 6.65±0.65). GLP-1 had no effect on the amplitude of the $[Ca^{2+}]_i$ oscillations at 1G (20 alpha-cells in 10 islets; n=6 donors). They were also unaffected by 1 μM insulin (42 alpha-cells from 4 islets; n=2 donors) or 10 μM Zn^{2+} (41 alpha-cells in 5 islets; n=2 donors). In an alpha-cell in an intact islet GLP-1 depolarized the cell by a few mV and reduced the peak voltage of the action potential by ~10 mV.
Conclusion: GLP-1 receptors are expressed at low levels in alpha cells. Blockade of insulin and SST signalling does not affect glucagon inhibition. GLP-1 effects on glucagon secretion can be mimicked by nanomolar concentrations of forskolin. These data suggest that GLP-1 principally acts by a direct effect on human alpha cells
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Effects of glucose, GLP-1 and adrenaline on alpha cell electrical activity and glucagon secretion from mouse pancreatic islets

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Background and aims: Pancreatic islet α-cells secrete glucagon during hypoglycaemia. Glucagon secretion is suppressed by elevation of glucose concentration and by the incretin hormone GLP-1 but stimulated by the stress hormone adrenaline. Like beta cells, alpha cells generate action potentials to allow influx of Ca2+ which in turn triggers exocytosis of glucagon-containing granules. Several ion channels are involved in this process. Both voltage gated Na+ channels and Ca2+ channels contribute to the upstroke of the action potentials (AP) and K+ channels are involved in repolarisation. Here we have analyzed the mechanism by which glucose, GLP-1 and adrenaline modulate glucagon secretion.
Materials and methods: Static incubation experiments using intact mouse islets were performed to measure glucagon secretion under treatment of different glucose concentrations as well as various reagents. Electrical activity and exocytosis was measured by patch-clamp measurements in alpha cells within intact mouse islets. In membrane potential recordings, alpha cells were identified by its active electrical activity when islets were exposed to 1 mmol/l glucose. In voltage clamp experiments, cells were infused with biocytin and later indentified by immunocytochemistry.
Results: Glucagon secretion is suppressed by 6 mmol/l glucose by ~50%. GLP-1 exerted similar effect while adrenaline stimulated the secretion of glucagon by ~2-fold. Mouse alpha cells exposed to 1 mM glucose fire overshooting APs starting at -51 mV with a peak of +5 mV. Glucose (6 mmol/l) depolarises the interspike voltage by ~4 mV and reduced the peak voltage by 6 mV. GLP-1 (100 nmol/l) did not detectably affect the interspike voltage but reduced spike height by ~2 mV. GLP-1 inhibited N-type Ca2+-channels by a PKA-dependent mechanism. Inhibition of N-type Ca2+-channels using ω-conotoxin mimicked the effects of glucose and GLP-1 on glucagon secretion. Inhibition of N-type Ca2+-channels by ω-conotoxin or GLP-1 inhibited α-cell exocytosis. Blockade of N-type Ca2+-channels reduced action potential amplitude by ~2 mV without affecting the interspike voltage. Adrenaline (10 μmol/l) depolarised the α-cell by ~10 mV and lowered the amplitude of APs by 20 mV. Glucagon secretion enhanced by adrenaline involved L-type Ca2+-channels and the cyclic AMP sensor Epac2.
Conclusion: We propose that glucose and GLP-1-induced suppression of glucagon secretion by inhibition of N-type Ca2+-channel activity. In the case of glucose, this effect is secondary to membrane depolarisation and voltage-dependent inactivation of the N-type Ca2+-channels. GLP-1 directly affects N-type Ca2+-channel gating by an effect not mediated by membrane depolarisation. In both cases, the resultant inhibition of N-type Ca2+-channel activity results in suppression of depolarisation-evoked exocytosis. Adrenaline by-passes this inhibitory effect by activation of Epac2 and L-type Ca2+-channels.
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PPAR delta positively regulates GLP-1 production by intestinal endocrine L cells and improves the oral glucose-induced insulin secretion in diabetic obese mice

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Background and aims: GLP-1 (Glucagon-Like Peptide-1) is an incretin hormone derived from the proglucagon gene transcription product and secreted by the intestinal L cells in response to dietary nutrients. GLP-1 stimulates glucose-dependent insulin secretion and insulin biosynthesis, inhibits glucagon secretion and gastric emptying and reduces food intake. In this study, we examined the regulation of GLP-1 production by PPARdelta/beta (Peroxisome Proliferator-Activated Receptor delta/beta) which is a nuclear receptor reported to improve islet function and to prevent weight gain.
Materials and methods: We used the murine and human L cell lines (respectively GLUtag and NCI-H716) activated or not with the synthetic PPARdelta ligands (GW501516 and GW0742). Wild type and diabetic ob/ob mice were also treated with these compounds.
Results: We show in the L cell lines that in vitro PPARdelta activation induces proglucagon gene expression in a dose and time dependant manner (up to 3 fold with 1μM of GW501516 at 24h) and increases to the same extent glucose and bile acid-induced GLP-1 release. Interestingly, we show that a 15 day oral treatment of wild type and ob/ob mice with GW501516 (once per day at 10 mg/kg) increases (3 fold increase compared to vehicle) GLP-1 secretion at 15 min after orally glucose tolerance test (O-GTT) but not after IP-GTT and that is correlated with the up-regulation of proglucagon gene expression in the ileum (2 fold increase) and the up-regulation of GLP-1 receptor expression in pancreas. In addition, we also observed in vivo and in vitro the positive effect of PPARdelta activation by another agonist GW0742. Moreover, GW501516 treatment in ob/ob mice decreases the basal insulin mRNA expression in the pancreas and basal insulin concentration in the plasma which could reflect the improvement of the glycemic control.
Conclusion: Altogether, these data suggest that the PPARdelta/beta activation potentiates the incretin system that is crucial in regulating postprandial glycemia and that pharmacological targeting of PPARdelta/beta may constitute a promising incretin-based strategy for the treatment of insulin resistance, diabetes and associated metabolic disorders.
Supported by: AMIDiab

PS 50 Incretins *in vivo*

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Effects of enterically coated, nutrient-containing pellets on glycaemia and incretin hormone release in patients with type 2 diabetes

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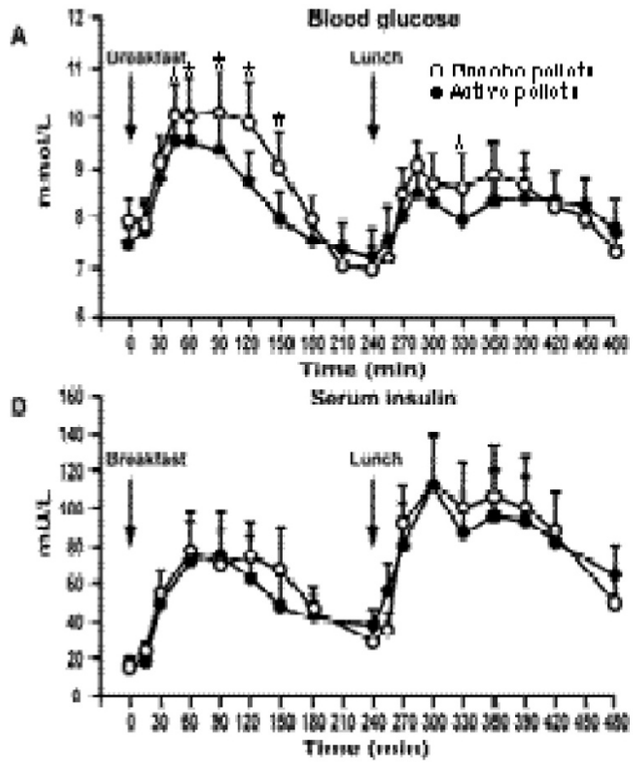
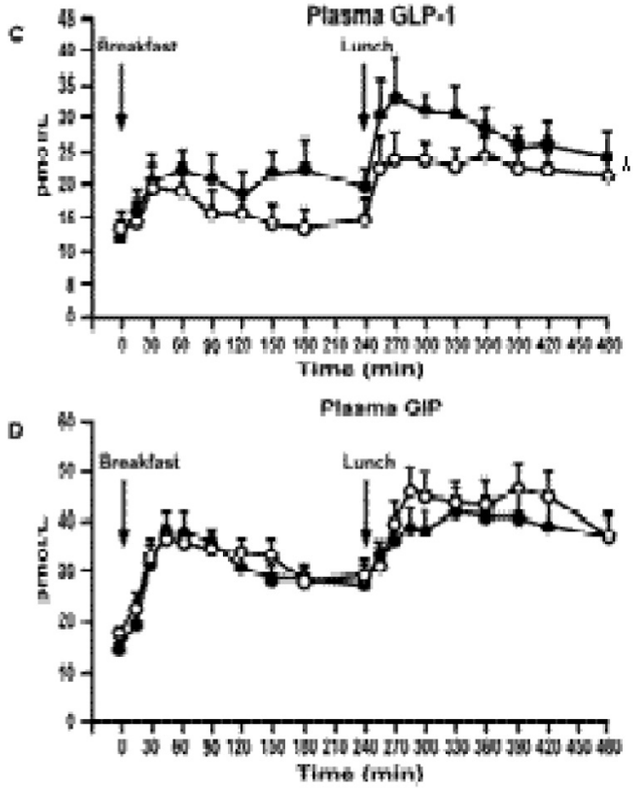
Background and aims: In principle, delivery of a small amount of nutrient to a long length of distal gut could be a potent stimulus for release of glucagon-like peptide-1 (GLP-1), with consequent improvements in glycaemic responses to subsequent meals. We evaluated the effects of enteric coated pellets designed for continuing release of small amounts of lauric acid along ileum and colon, on GLP-1 secretion and the glycaemic response, after both breakfast and a subsequent lunch.
Materials and methods: Ten patients with well controlled type 2 diabetes (glycated haemoglobin 5.9 ± 0.2 %), managed by diet alone, were studied on two separate days. On each study day, they ingested either 10 g active pellets (47 % lauric acid by weight, or ~40 kcal) or placebo pellets at T = 0 min during breakfast (71 g carbohydrate, 4.3 g protein, 12 g fat, 415 kcal). A second meal (lunch; 89 g carbohydrate, 29 g protein, 26 g fat, 708 kcal) was consumed at T = 240 min. Blood was sampled for measurement of blood glucose, serum insulin, and plasma total GLP-1 and glucose-dependent insulinotropic polypeptide (GIP).
Results: Data are shown as mean ± standard error. After active pellets, blood glucose concentrations were lower, and plasma GLP-1 concentrations higher, than after placebo, following both breakfast and lunch (*P < 0.05 in the figure). In contrast, the rises in insulin and GIP after breakfast and lunch did not differ between active pellets and placebo.
Conclusion: Enteric coated pellets which sustain release of lauric acid along distal small intestine and colon diminish the glycaemic response to both breakfast and lunch in type 2 diabetes, associated with stimulation of GLP-1, establishing that exposure of the distal gut to a small quantity of nutrient can have substantial effects on GLP-1 release and hence glycaemic control.



Supported by: NHMRC



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Degradation of GIP but not of GLP-1 is reduced after protein ingestion in obese subjects

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Background and aims: The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are released after meal ingestion and potentiate glucose-stimulated insulin secretion. Both hormones are rapidly degraded by the enzyme DPP-4 (dipeptidyl peptidase 4). This major site of degradation is thought to be the gut wall, where we previously showed in animal studies that DPP-4 activity varies depending on macronutrient ingestion, being reduced by protein. We previously showed that secretion and metabolism of the incretin hormones after mixed meal and oral glucose is different in obese versus lean subjects. Thus, GLP-1 but not GIP secretion is lower in obesity after meal ingestion and oral glucose, whereas GIP but not GLP-1 metabolism is increased in obesity after meal ingestion. In this study, we have proceeded and examined GIP and GLP-1 secretion and metabolism after oral ingestion of pure protein in lean versus obese subjects.
Materials and methods: The study was undertaken in healthy male human volunteers that were either lean (BMI 20-25kg/m^2; n=12) or obese (BMI 30-35kg/m^2; n=12). After an overnight fast, the subjects at three different occasions consumed 2.22g protein mix (Promax protein 85^R)/kg b wt. Blood samples were collected before and up to five hours after intake of each macronutrient for measurements of total(t) and intact(i) GLP-1 and GIP. Hormone responses were assessed as suprabasal AUC300 over the entire 300 minutes study period.
Results: Both GIP and GLP-1 secretion increased following ingestion of protein, as reflected by increased concentrations of tGLP-1 and tGIP. Whereas the secretion of GIP was markedly lower in the obese subjects (AUC_{tGIP} 10.0±2.8 vs. 18.4±2.7 pmol/l; P=0.046), there was no difference between obese and lean subjects in AUC_{iGIP}. This suggests that degradation of GIP is reduced after protein ingestion in obese subjects. In contrast, GLP-1 did not show any differences between lean and obese after protein ingestion for either

intact or total levels, suggesting that secretion and degradation of GLP-1 are not affected by obesity.
Conclusion: Our results show a reduced GIP but a normal GLP-1 secretion after protein ingestion in obese subjects in association with a reduced rate of GIP degradation but a normal degree of GLP-1 degradation. This resulted in normal concentrations of intact forms of the two incretins after protein ingestion in obesity. The reduced degradation of GIP after protein ingestion in obese subjects may be the result of decreased DPP-4 activity after protein in the proximal portion of the gut.
Supported by: The Swedish Research Council, Region Skåne, Faculty of Medicine, Lund University

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Short-term intervention with steroid hormone, relative physical inactivity and high calorie diet in healthy subjects results in increased postprandial GIP and glucagon responses
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Background and aims: The incretin hormone glucose-dependent insulinotropic polypeptide (GIP) has been proposed as a link between consumption of high fat diets and the development of obesity, insulin resistance, hyperinsulinaemia and type 2 diabetes mellitus (T2DM). In obese subjects the GIP response to a mixed meal is reported to be increased perhaps contributing to postprandial hyperinsulinaemia. Additionally GIP has been shown to stimulate glucagon secretion. Postprandial responses of the other incretin hormone glucagon-like peptide-1 (GLP-1) has been reported to be decreased in obese individuals as well as in patients with T2DM. In the present study we evaluated the impact of disruption of insulin sensitivity on postprandial GIP, GLP-1 and glucagon responses in healthy young male subjects without any risk factors for diabetes.
Materials and methods: Postprandial GIP, GLP-1 and glucagon responses were measured using a 520 kcal-liquid meal test (58 g carbohydrate, 28 g fat and 10 g protein) in 10 healthy Caucasian male subjects without family history of diabetes (age: 23.9±3.1 years (mean±SD); BMI: 24.1±1.7 kg/m^2; fasting plasma glucose: 4.9±0.3 mM, HbA_1c: 5.4±0.1%) before and after induction of insulin resistance using high calorie diet, relative physical inactivity and administration of prednisolone (37.5 mg/day) for 10 days.
Results: The intervention had a significant impact on insulin resistance according to the homeostatic model assessment (1.4±0.1 *vs.* 2.3±0.4, p=0.02) without affecting body weight. In line with this, fasting insulin levels (36±3 *vs.* 61±6 pM, p=0.03) and insulin responses (as assessed by AUC) increased following the intervention (30±6 *vs.* 59±16 nM·4h, p=0.02). The impaired insulin sensitivity had no impact on postprandial GLP-1 responses (1.5±1 *vs.* 2.0±1 nM·4h, p=0.56), but postprandial GIP responses rose significantly following induction of insulin resistance (10.6±1.3 *vs.* 14.1±1.5 nM·4h, p=0.03) as well as postprandial glucagon responses (1.6±1.5 *vs.* 2.4±3.2 nM·4h, p=0.03).
Conclusion: Our data show that induction of insulin resistance using prednisolone, high calorie diet and relative physical inactivity results in increased postprandial GIP responses suggesting that increased GIP secretion observed in conditions characterised by insulin resistance may occur as a consequence of insulin resistance rather than being a primary pathogenetic trait. Additionally, our data suggest that hyperglucagonaemia (observed in obese individuals with type 2 diabetes) may be a consequence of insulin resistance, while insulin resistance not seems to be directly responsible for the reduced postprandial GLP-1 responses observed in individuals with type 2 diabetes.
Supported by: an EFSD/Novartis grant

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The incretin effect is apparent after lipid challenge in healthy humans
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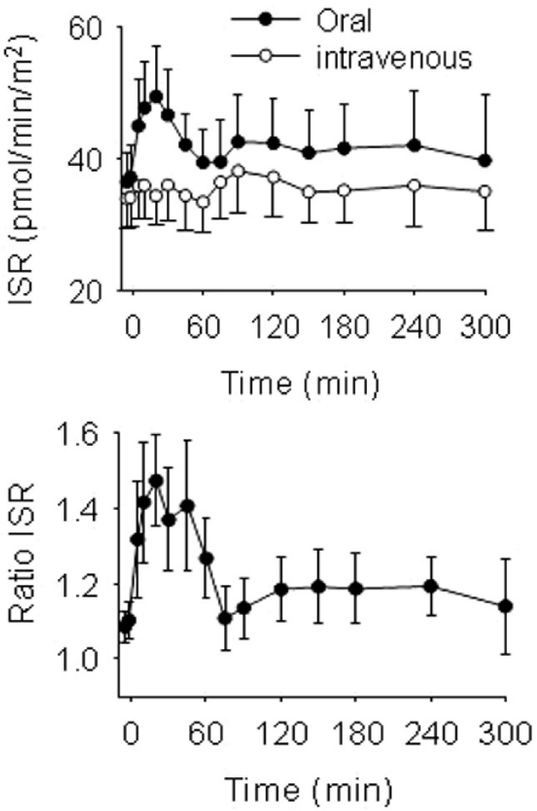
Background and aims: It is well known that oral glucose gives rise to a greater insulin response than an isoglycaemic intravenous (iv) glucose infusion, due to the incretin effect. We examined whether an incretin effect exists also after lipid ingestion.
Materials and methods: After an overnight fast, 12 healthy male volunteers (age 20-30 years; BMI 20-25 kg/m^2) received lipids (intralipid^R) orally (0.6 g/kg) or iv (infusion rate min 0-30 0.15g/min, min 30-60 0.30g/min and min 60-90 0.45g/min, designed to mimic plasma triglyceride (TG) responses to the oral load). Blood samples were taken over 300 min for analysis of TG, glucose, insulin, C-peptide and intact and total concentrations of glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP).
Results: Plasma glucose did not rise during lipid administration, whereas plasma TG levels rose similarly during oral and iv lipid (peak at 180 min of 1.0±0.2 mmol/l for both). Plasma insulin and C-peptide were unchanged during iv lipid but rose after oral lipid, the difference being largest during the first 60 min. Suprabasal 0-60 min AUC for insulin was 0.46±0.10 nmol/lx60 min after oral lipid vs. -0.11±0.14 after iv lipid (P=0.03); corresponding values for C-peptide were 2.45±0.45 vs. 0.43±0.32 nmol/lx60 min (P=0.001). Figure (left panel) shows the insulin secretory rate (ISR) during the 300 min tests and (right panel) the ratio of ISR after oral vs. iv lipid as estimated from C-peptide data, illustrating the larger insulin secretory response to oral vs iv lipid with matching TG levels. Plasma intact and total GLP-1 and GIP were unchanged during iv but rose after oral lipid. Suprabasal 0-60 min intact GLP-1 was 243±53 pmol/lx60 min after oral lipid vs. -9±5 after iv lipid (P<0.001); corresponding values for intact GIP were 302±118 vs. 9±53 pmol/lx60 min; P=0.031). Suprabasal 60 min AUC for ISR correlated to corresponding AUC for both intact GLP-1 (r=0.42; P=0.043) and intact GIP (r=0.42; P=0.047).
Conclusion: Oral vs iv lipid administration to matching TG levels reveals that oral lipid elicits a clear incretin effect with a pronounced stimulation of insulin secretion during the first 60 min. Since oral lipid as well stimulates the release of the two incretins and the increase in intact (insulinotropic) GLP-1 and GIP correlates with insulin secretion, we conclude that an incretin effect induced by the classical incretin hormones also exists after oral lipid ingestion in healthy humans.



Supported by: Swedish Research Council

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Effect of GLP-1 and exendin-4 treatment on glucose and fat metabolism, in obese state

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Background and aims: Obesity is often associated with hypertension, cardiovascular disease and diabetes. GLP-1, incretin with insulin-independent antidiabetic actions, and its homologous exendine-4, both have shown positive effects upon the glucose metabolism of extrapancreatic tissues participating in the hexose homeostasis. Here we studied the effect of GLP-1 and Ex-4 on GLUT-4 expression and other parameters, in fat and muscle of an obesity (Ob) rat model, compared to normal (N).
Materials and methods: Ob was developed in male Wistar rats by chronic feeding -5 weeks- with a daily intake of standard chow combined with a "cafeteria diet" (65 % energy from lipids). The N group was fed with standard chow and water *ad libitum* (8 % energy as fat). Although weight was not different between N and Ob, the Ob model showed fasting plasma glucose, triglycerides (153±13 mg/dl, n=11) and cholesterol (92±4 mg/dl, n=12) higher than normal (overall mean: 37±5 % Δ N-rats, $p<0.02$); no significant differences with N were detected in insulin or GLP-1 -by RIA-. Ob and N were 3-days treated -through an osmotic pump- with saline (control), GLP-1 (0.86 nmol/kg/h) or Ex-4 (0.1nmol/kg/h). Blood samples were taken before and by the end of the treatment for plasma measurements. In epididimal fat and *soleous* muscles, we studied: GLUT-4 expression -mRNA by RT-PCR, and protein by Western blot-, isolated adipocytes glucose transport (GT) -2-deoxi-D-[1,2-^{3}H]glucose uptake- and muscle glycogen synthase *a* activity (GS*a*) -UDP-glucose into glycogen-.
Results: In muscle of Ob (n=5-7 rats), GLUT-4-protein was lower than in N (71±4 % N-control, $p<0.01$) while mRNA was higher (1.81±0.04 times N-control, $p<0.001$); GLP-1 did not modify mRNA, but increased the protein, to a value (180±14 % Ob-control, $p<0.01$) even higher (128±7 % N-control, $p<0.02$) than that in N (100±13 % N-control, n=6); Ex-4, like GLP-1, failed to modify mRNA, but stimulated the GLUT-4-protein (129±6 % Ob-control, $p<0.05$) to N levels (92±4 % N-control). GS*a* in Ob (1.30±0.16 U/g protein) was lower ($p<0.001$) than in N; GLP-1 did not affect the GS*a*, while Ex-4 induced a clear increase (175±20 Ob-control, $p<0.02$) toward normalization; no apparent effect was detected in N after GLP-1 or Ex-4. In fat of Ob (n=5-9), GLUT-4-mRNA was lower than normal (0.39±0.05 times N-control, $p<0.001$), without differences in the protein; either GLP-1 or Ex-4 reduced GLUT-4-mRNA even further (GLP-1: 0.29±0.05 times Ob-control; Ex-4: 0.65±0.15; both $p<0.01$), as previously observed in N rats; both GLP-1 and Ex-4 exerted a slight but clear increase in GLUT-4-protein (137±9 % Ob-control and 118±5 %, respectively, both $p<0.02$). GT in Ob (6.7±0.7 fmol/10^5 cells) was lower ($p<0.001$) than in N (15.1±1.6 fmol/10^5 cells); GLP-1 and Ex-4 increased the value (159±10 % Ob-control and 178±18 %, respectively, both $p<0.01$) toward normalization (overall mean: 74±8 % N-control). In Ob, Ex-4 highly reduced to normalization the triglycerides (86±3 mg/dl, $p<0.01$ *vs* Ob-control) and cholesterol values (75±3 mg/dl, $p<0.01$ *vs* Ob-control), while GLP-1 only decreased triglycerides to normalization (103±9 mg/dl, $p<0.05$ *vs* Ob-control).
Conclusion: In obese state, both GLP-1 and Ex-4 could exert a beneficial effect on its deleterious glucose metabolism, perhaps by their increasing action upon the muscle and fat glucostransporter translation process, together with a normalizing effect on the impaired lipid metabolism.
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The separate and combined impact of the intestinal hormones GIP, GLP-1 and GLP-2 on glucagon secretion in type 2 diabetes

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Background and aims: Type 2 diabetes mellitus (T2DM) is associated with reduced suppression of glucagon during oral glucose tolerance test (OGTT) whereas isoglycaemic intravenous (*iv*) glucose infusion (IIGI) results in normal glucagon suppression in these patients. We aimed to evaluate the role of the intestinal hormones glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) in this discrepancy.
Materials and methods: Glucagon responses were measured during a 3-hour 50 g-OGTT (day a) and a corresponding IIGI (day b) in 10 patients with T2DM (age (mean±SEM): 51±3 years; BMI: 33±2 kg/m^2; HbA_1c: 6.5±0.2%). During four additional IIGIs, GIP (day c), GLP-1 (day d), GLP-2 (day e) and a combination of the three intestinal hormones (day f), respectively, were infused intravenously to mimic postprandial responses.
Results: Isoglycaemia during all six study days was obtained. As expected, no suppression of glucagon occurred during the initial phase of the OGTT, whereas significant ($p<0.05$) suppression of glucagon during the first 30 minutes of the IIGI (day b) was observed. As illustrated in Fig. 1, the glucagon response during the IIGI+GIP+GLP-1+GLP-2 (day f) equalled the inappropriate glucagon response to OGTT (p=NS). The separate GIP infusion (day d) elicited significant hypersecretion of glucagon, whereas GLP-1 infusion (day d) resulted in potentiation of glucagon suppression during IIGI. IIGI+GLP-2 infusion resulted in a glucagon response in the mid-range between the inappropriate glucagon response to OGTT and the preserved suppression during IIGI.
Conclusion: Our results indicate that the intestinal hormones, GIP, GLP-1 and GLP-2, may play a role in the inappropriate glucagon response to orally ingested glucose in T2DM with GIP acting as a strong glucagonotropic substance.

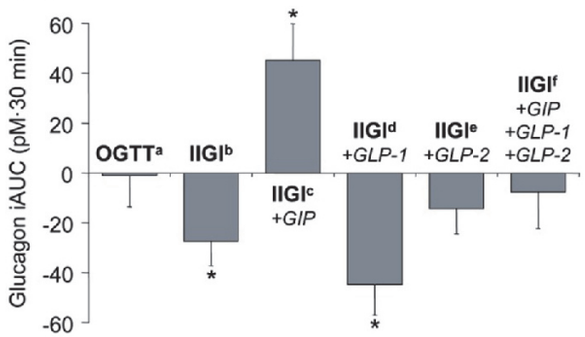

Figure 1. Initial glucagon responses following OGTT and IIGIs in patients with T2DM expressed as incremental AUCs (mean±SEM). Asterisks indicate significant difference ($p<0.05$) from 0.

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The contribution of GLP-1 to the enteroinsulinar axis in type 2 diabetes mellitus

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Background: The gut born hormones GLP-1 and GIP augment glucose-dependently the postprandial (PP) insulin release from the pancreatic B-cell, mediating the so-called incretin effect which constitutes the difference between the postprandial (PP) and the isoglycemic fasting insulin response. In addition, GLP-1 reduces glucagon. In T2DM, the incretin effect is reduced and plasma glucagon increased. A defect of the GIP action has been suggested in T2DM. However, the contribution of either hormone in T2DM is not known.
Aim: To analyze the contribution of endogenous GLP-1, we examined the effect of a duodenally perfused meal on pancreatic insulin and glucagon secretion using the specific GLP-1 receptor antagonist exendin(9-39) (Ex-9).
Methods: 12 patients with T2DM (age 60±2, BMI 28.1±1.2, HbA1c 6.4±0.3) and 12 healthy subjects (HS) participated in 3 study days in random order. Plasma insulin and glucagon concentrations were measured during a 3 hour hyperglycemic clamp at 180mg/dl using IV glucose. 30 min prior the clamps, a duodenal meal perfusion (2.03 kcal/min, 77% lipid, 23% glucose) was initiated and continued throughout the study on 2 days. This was accompanied by IV infusion of Ex-9 (600 pmol/kg/min) or saline, respectively. A third day with duodenal perfusion of saline served as isoglycemic fasting control (IF) to calculate the incretin effect. The acute insulin response (AIR) to hyperglycemia and the sustained responses of plasma insulin (SIR) and glucagon (SGR) were calculated during the first 10min and between 60 and 120min of the hyperglycemic clamp as incremental AUC (see table).
Results: In HS, AIR and SIR during the duodenal meal (PP) were markedly increased compared to isoglycemic fasting conditions (IF) corresponding to an incretin effect of 0.34±0.1 and 5.1±0.8 mU/ml*min, respectively. In T2DM,

both AIR and SIR were significantly lower compared to HS and the incretin effect was significantly reduced (0.13±0.04 and 1.9±0.7 mU/ml*min, p<0.05 vs HS). Ex-9 reduced PP AIR and SIR both in HS and T2DM. Also with Ex-9, PP AIR and SIR remained significantly elevated compared to IF both in HS and T2DM corresponding to a GLP-1 independent incretin effect in T2DM of 0.08±0.03 (AIR) and 0.60±0.25 (SIR) mU/ml*min, thus representing 79% and 51% of PP insulin response, respectively. Plasma glucagon concentrations were markedly elevated in T2DM. Ex-9 significantly increased SGR in both, HS and T2DM.

Conclusion: Both the acute and sustained postprandial response to hyperglycemia is largely mediated by gut hormones. This incretin effect is clearly reduced in T2DM compared to HS. However, GLP-1 and non-GLP-1 incretins contribute equally to the incretin effect both in HS and T2DM. We suggest GIP to account for the remaining incretin effect also in T2DM. As in HS endogenous GLP-1 contributes largely to the postprandial suppression of plasma glucagon. We conclude that although pancreatic islet cell secretion is disturbed in T2DM, the enteroinsulinar axis seems to be intact also in T2DM.

	HS			T2DM		
	PP+SAL	PP+Ex9	IF	PP+SAL	PP+Ex-9	IF
AIR (mU/ml*min)	0.60±1.5	0.38±0.08*#	0.25±0.08	0.16±0.05	0.10±0.04*#	0.02±0.02
SIR (mU/ml*min)	6.4±1.0	3.5±0.4*#	1.3±0.5	2.5±0.8	1.2±0.3*#	0.6±0.2
SGR (ng/ml*min)	-0.8±0.3	-0.3±0.26*#	-1.1±0.2	0.05±0.2	0.9±0.2*#	-0.9±0.2

AUC, Mean±SEM. ANOVA: *P<0.05 vs SAL, #P<0.05 vs IF

Supported by: DFG

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Insulin resistance and glucose intolerance independently reduce the incretin effect via a beta cell defect

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Background and aims: The incretin effect is impaired in type 2 diabetes. We wanted to evaluate the separate impact of insulin resistance and glucose intolerance on the incretin effect and whether changes in the incretin effect were associated with β-cell defects.

Materials and methods: 21 healthy 1st degree relatives to type 2 diabetes patients with normal glucose tolerance underwent a 75g OGTT on day 1 and an isoglycemic i.v. glucose test on day 2. The two tests were performed before and after 5 days treatment with 2mg dexamethasone bid. Insulin, C-peptide, GIP and GLP-1 were measured during the 4 tests. The incretin effect was estimated by relating the incremental insulin response during the OGTT to the incremental insulin response during the i.v. glucose infusion. Furthermore, the insulin secretion rates during the i.v. glucose infusions were plotted against ambient P-glucose, and the slopes of these linear relations were used as an index of the β-cell glucose sensitivity, which is known to be impaired already in the early stages of type 2 diabetes. To relate the β-cell glucose sensitivity to the ambient insulin resistance the disposition index was calculated by multiplying β-cell glucose sensitivity with $1/HOMA_{IR}$.

Results: The dexamethasone treatment increased insulin resistance in all 21 subjects, and 11 subjects in addition to insulin resistance also developed glucose intolerance (IGT) (10 subjects remained glucose tolerant (NGT)). The incretin effect was in the NGT and IGT group 71±3.2 and 67±4.6% before and 58±5.2 and 32±8.8% after treatment, respectively. There was no difference in the incretin effect between groups at baseline but a significant difference after treatment (P < 0.05). A multiple regression analysis of pooled data from the two groups related the changes in incretin effect (Δincretin effect) to changes in insulin resistance ($\Delta HOMA_{IR}$) and glucose tolerance (delta 2-hour P-glucose during OGTT, ΔPG_{120}). ΔPG_{120} and $\Delta HOMA_{IR}$ were negatively and independently correlated with Δincretin effect (P < 0.05), and accounted for 45% of the overall variation in Δincretin effect. The disposition index was 1.7±0.20 and 1.4±0.17 pmol kg^{-1} min^{-1} mM^{-1} in the NGT and IGT groups, respectively (P = NS). After the dexamethasone treatment this index was 1.3±0.19 in the NGT group and 0.9±0.12 pmol kg^{-1} min^{-1} mM^{-1} in the IGT group, respectively (P < 0.05). The reduction in the disposition index in the NGT group was not significant (P =NS). Responses of GLP-1 and GIP did not differ between groups before or after dexamethasone during the OGTT.

Conclusion: Insulin resistance and glucose intolerance contribute independently to the reduced incretin effect seen in type 2 diabetes. In addition we find that the incretin effect is reduced very early in NGT insulin resistant people, before the β-cell sensitivity to i.v. glucose is affected, and deteriorates further when β-cell function declines in people with IGT. This points to an early, specific β-cell defect in the action of the incretin hormones before the development of overt type 2 diabetes.

Supported by: The Novo Nordisk Foundation

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GIP controls human core circadian genes indicating an integrative role in food-regulated metabolism

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Background and aims: Adaptation of metabolism to circadian rhythms is critical for its proper function. Rhythmically expressed peripheral clock genes are regulated by food intake but detailed mechanisms are unknown. We investigated the role of the food induced hormones, GIP, insulin and glucose on expression of core circadian genes in human adipose tissue.

Materials and methods: 17 overweight healthy humans (BMI: 28-40 kg/m2, age: 30-65 y) with normal glucose tolerance were infused with placebo or GIP in physiological doses for 4h i.v. either alone, or during euglycemic or hyperglycemic hyperinsulinemic clamps. Biopsies were taken from subcutaneous adipose tissue before and after treatment. Total RNA was isolated from all biopsies and transcribed into cDNA. Expression patterns of circadian genes were analysed by hybridisation to a total number 100 Agilent 60-mer Whole Human Genome (4x44K) single-color DNA microarrays and results confirmed by quantitative RT-PCR. Statistical analysis of microarray data was performed with Agilent GeneSpring GX software.

Results: The expression of REV ERBalpha (NR1D1) was regulted during the 4 h treatment even under placebo conditions and decreased significantly about 4 fold. The expression of other circadian genes like PER2, PER3, TEF and DBP did not change significantly. Neither insulin nor glucose affected clock gene expression in euglycemic or hyperglycemic hyperinsulinemic clamps. However, GIP either alone or combined with insulin and glucose, significantly enhanced the circadian decrease of the core clock genes REV ERBalpha 6.5 - 9 fold as well as PER2, PER3 and the clock output genes TEF and DBP between 1.5 - 2.5 fold. These genes were among the most significantly GIP-regulated genes even after correction for multiple testing (Benjamini Hochberg). Additional correlation analysis showed a disintegration of clock gene correlations among each other as a result to GIP administration. The decrease was not reproduced in a mouse model upon treatment with GIP at different circadian time points pointing to species specific differences.

Conclusion: We identify GIP as a powerful regulator of the core clock gene machinery in human adipose tissue in vivo which provides the missing link from food intake to peripheral clock gene regulation. Since i.e. REV ERBalpha is involved in regulation of glucose, lipid and cholesterol metabolism, fat cell differentiation and inflammatory processes, the disruption of circadian rhythms by GIP may explain its obesogenic and fatty liver inducing properties.

Supported by: DFG

PS 51 Clinical insulin secretion - methods and associations

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Dynamic beta cell function in young type 2 diabetes patients (15-34 years) in the Diabetes Incidence Study in Sweden register

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Background and aims: Simple methods for evaluation of dynamic beta-cell function in epidemiological and clinical studies of type 2 diabetic patients (T2D) are needed. The first phase insulin response after intravenous (iv) glucose is diminished whereas the insulin response after non-glucose stimulation, i.e. iv arginine is less dependent on prevailing glucose levels. The objective of this study was to compare these methods for assessment of dynamic beta-cell function in young T2D with different disease duration and treatments.
Materials and methods: 54 T2D patients from the Diabetes Incidence Study in Sweden (DISS) and 23 healthy controls; group-wise matched for age (35±5.4 vs 40±5.8 years), sex (M/F 33/21 vs. 12/11) and BMI (33±5.7 vs. 33±4.5 kg/m^2) were included in a cross-sectional study. Beta-cell function was assessed by iv pulses of arginine 5g followed after 30 min by iv glucose 0.3g/kg. The acute insulin and c-peptide response to arginine (AIR_{arg} and $A_{c\text{-}pep}R_{arg}$) and to glucose (AIR_{glu} and $A_{c\text{-}pep}R_{glu}$) was calculated as the mean of the three highest levels of insulin and c-peptide obtained during 5 minutes minus the plasma level of insulin and c-peptide at baseline.
Results: Fasting p-glucose at baseline was 7.5 mmol/L in T2D and 5.5 mmol/L in healthy controls. AIR_{glu} and $A_{c\text{-}pep}R_{glu}$ were reduced approx 90% in T2D and AIR_{arg} and $A_{c\text{-}pep}R_{arg}$ were reduced approx 30% in T2D (7.6±24 vs.93±60, p<0.05; 0.5±1.3 vs. 4.2±2.4, p<0.05 and 51±45 vs.72±43, p= 0.07 and 2.4±1.6 vs 3.2±1.4, p=0.06)(compared to the healthy controls). AIR_{glu} and $A_{c\text{-}pep}R_{glu}$ but not AIR_{arg} and $A_{c\text{-}pep}R_{arg}$ responses was negatively correlated with fasting p-glucose at baseline (r= -0.52, r=-0.48 p<0.05 and r=0.10 and r=0.15, ns). AIR_{arg} and $A_{c\text{-}pep}R_{arg}$ disciminated patients with different disease duration better than AIR_{glu} and $A_{c\text{-}pep}R_{glu}$ as displayed in Table. In addition, AIR_{glu} and $A_{c\text{-}pep}R_{glu}$ was higher in the diet-treated group compared to other treatments, while AIR_{arg} and $A_{c\text{-}pep}R_{arg}$ were decreased in insulin-treated patients compared to other treatments groups (see Table).
Conclusion: AIR_{arg} and $A_{c\text{-}pep}R_{arg}$ were less reduced than AIR_{glu} and $A_{c\text{-}pep}R_{glu}$ in young type 2 diabetic patients and discriminated better between groups with different disease duration. AIR_{arg} and $A_{c\text{-}pep}R_{arg}$ was also less dependent on baseline plasma glucose levels. AIR_{arg} and $A_{c\text{-}pep}R_{arg}$ may be used epidemiological and clinical studies to select patients suitable for drugs affecting dynamic beta-cell function.

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The graded glucose infusion is comparable to the hyperglycaemic clamp as a tool for measurement of glucose-dependent insulin secretion

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Background and aims: The hyperglycemic clamp (HGC), considered the gold standard method for measurement of glucose-dependent insulin secretion (GDIS) is technically resource intensive. In contrast, the Graded Glucose Infusion (GGI) is a simpler, but less well-characterized method. We tested the hypothesis that the GGI is comparable to the HGC as a tool to measure changes in GDIS, by performing a randomized, double-blind, placebo-controlled experiment that directly compared the GGI and the HGC, using exenatide as a probe.
Materials and methods: Following an overnight fast, 18 non-obese healthy subjects (age 29 ± 8 yrs, BMI 23.5 ± 2.3 kg/m^2, basal fasting glucose 4.50 ± 0.24 mmol/L) received 5µg exenatide s/c or matched placebo, immediately prior to the procedures. Each subject underwent a GGI (glucose infusion rates (GIR) of 2, 4, 6 and 12mg/kg/min, 40 minute periods each) and a HGC (glycemia maintained at 11.1 mmol/L for 120 minutes; steady-state (SS) 90 to 120 minutes) on separate days, in a randomized crossover design.
Results: Data are presented as Mean ± SEM, exenatide vs placebo. HGC: At matched SS plasma glucose (10.80 ± 0.11 vs 10.71 ± 0.13 mmol/L), significant differences (p<0.001 for all comparisons) were observed in GIR (1.05 ± 0.09 vs 0.45 ± 0.07 mmol/kg/min), plasma insulin (4111.44 ± 303.50 vs 322.94 ± 225.02 pmol/L), C-peptide (9.76 ± 0.59 vs 2.06 ± 0.47 nmol/L), and insulin secretion rates (ISR) (1398.6 ± 99.9 vs 333.0 ± 66.6 pmol/min). GGI: At matched GIR, significant (p<0.001 for all comparisons) differences were observed in ambient plasma glucose levels (G) (7.72 ± 0.59 vs 13.19 ± 0.59 mmol/L), ISR (1465.2 ± 166.5 vs 699.3 ±166.5 pmol/min), and Φ (slope of ISR/G: 4.2 ± 0.17 vs 1.89 ± 0.15). Both methods were compared using the only common end-point, the ratio of ISR to G. The ISR/G for exenatide in the GGI was comparable to that in the HGC (2.95 ± 0.29 vs 2.14 ± 0.15 respectively). The GGI was operationally simpler than the HGC.
Conclusion: This study demonstrates for the first time in a head to head setting, that the GGI is comparable to the HGC in its ability to detect clinically relevant changes in GDIS, while being less resource intensive. Our data indicate that the GGI is a sensitive, reliable and feasible tool for measurement of GDIS effects in healthy subjects.

Acute insulin (AIR, µU/ml) and c-peptide(Ac-pepR, nmol/L) responses after iv glucose and iv arginine

Diabetes duration	**<5 yrs** n= 11	**5-8 yrs** n=20	**>8 yrs** n=23	**p-value**	
AIRglu	8.6±38	10.4±26	4.6±8.4	ns	
Ac-pepRglu	0.4±2.0	0.7±1.5	0.3±0.7	ns	
AIRarg	83±52	57±51	31±20	p<0.05 >8 years vs. 5-8 and <5 years	
Ac-pepRarg	3.6±1.9	2.8±1.8	1.6±0.7	p<0.05 >8 years vs. 5-8 years	
Antidiabetic treatment	**Diet** n=10	**Tablets** n=22	**Tablets+insulin** n=12	**Insulins only** n=10	**p-value**
AIRglu	26.5±42	4.3±18	2.3±15	2.0±3.8	p<0.05 diet vs. other treatments
Ac-pepRglu	1.4±2.4	0.22±1.0	0.44±0.9	0.16±0.37	p<0.05 diet vs. other treatments
AIRarg	67±62	59±48	50±27	18±6.6	p<0.05 insulin vs. other treatments
Ac-pepRarg	2.9±2.0	2.8±1.8	2.5±1.2	1.2±0.5	p<0.05 insulin vs. other treatments

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A novel tool to measure changes in glucose-dependent insulin secretion in healthy subjects

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Background and aims: The Meal Tolerance Test (MTT) can detect changes in prandial glucose-dependent insulin secretion (GDIS) in Type 2 Diabetes Mellitus (T2DM) subjects; however, it is less suitable as a tool in healthy volunteers (HV), where effect sizes are small, and variability high. We hypothesized that changes in GDIS can be detected in HV, if the MTT is conducted in a milieu simulating the hyperglycemia of T2DM. We tested the hypothesis by performing a randomized, double-blind, placebo controlled, crossover study that measured GDIS effects of the DPP4 inhibitor sitagliptin, in the setting of a MTT superimposed on stable hyperglycemia achieved by a hyperglycemic clamp (HGC) (HGC-MTT).
Materials and methods: Following an overnight fast, and a single dose of sitagliptin 100mg or matched placebo, 12 healthy non-obese subjects (age 27 ± 6 yrs., BMI 20.5 ± 1.3 kg/m^2) underwent a HGC at target glycemia of 8.9 mmol/L for 370 minutes (mins.); a standardized liquid meal was administered at 180 mins, and consumed over 10 mins.
Results: Data are presented as Mean ± SEM, sitagliptin vs placebo. Premeal (120-180mins.): At matched plasma glucose (8.79 ± 0.05 vs 8.95 ± 0.06 mmol/L), significant ($p<0.001$ for all between group comparisons) differences were observed in glucose infusion rates (GIR) (0.72 ± 0.03 vs 0.58 ± 0.03 mmol/kg/min), active GLP-1 (1.7 ± 0.1 vs 1.2 ± 0.1 pmol/L), plasma insulin (1279.26 ± 228.49 vs 752.84 ± 231.96 pmol/L) and insulin secretion rates (ISR) (1598.4 ± 133.2 vs 1065.6 ± 133.2 pmol/min). Post-meal (190-340mins.)**:** At matched plasma glucose (9.34 ± 0.03 vs 9.26 ± 0.04 mmol/L), further significant ($p<0.001$ for all parameters) increments over pre-meal were seen in all parameters in both groups, and significant ($p<0.001$ for all between group comparisons) differences were observed in GIR (0.83 ± 0.02 vs 0.76 ± 0.02 mmol/kg/min), active GLP-1 (6.4 ± 0.6 vs 3.1 ± 0.7 pmol/L), plasma insulin (5809.49 ± 988.97 vs 3833.64 ± 993.13 pmol/L) and ISR (3230.1 ± 266.4 vs 2664.0 ± 266.4 pmol/min).
Conclusion: Our data demonstrate for the first time in HV that the GLP-1 stabilizing property of sitagliptin has significant GDIS effects in the preprandial state, with further significant augmentation in the prandial state. The HGC-MTT appears to be a novel reliable tool for measurement of preprandial and prandial GDIS in a single experiment in a single dose setting in HV.

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Differences in insulin release between long term type 2 diabetes mellitus and NGT - a model of discovery of changes in proteins using liquid chromatography-mass spectrometry (LC-MS)

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Background and aims: Proteomics-based candidate biomarker discovery efforts have gained significant attention due to the power of these technologies for analyzing complex protein mixtures and their potential for identifying novel markers indicative of disease. The aim of this project is to apply advanced quantitative proteomic methodology to quantify relative changes in protein levels in serum samples from individuals with normal glucose tolerance (NGT) and long term type 2 diabetes (T2DM) investigated with hyperglycaemic.
Materials and methods: 13 men 7 women were followed for 10 years, with these baseline characteristics: age 58,4 (6,1) (mean (SD)) years, diabetes duration 7,0 (3,0) years, HbA1c 8,5 (1,6)%, BMI 25,8 (2,7) kg/m2, weight 76,6 (10,3) kg and anti-GAD negative. Hyperglycaemic clamp was preformed in all with glucose increase of + 7.2 (1.10) mmol/l during two hours, followed by a bolus of 5 mg iv arginine stimulation. For comparison, the hyperglycaemic clamp with arginine was also performed in seven aged-matched healthy volunteers. Our approach uses an enzymatic 18O stable isotope labeling procedure followed by liquid chromatography- mass spectrometry (LC-MS) to directly detect and quantitatively compare proteins present in patients with T2DM to be compared with persons with NGT during the hyperglycaemic clamp. With this approach, we can rapidly identify and measure difference in expression levels for thousands of peptides in a single analysis. So far only insulin secretion data from the hyperglycemic clamp have been analyzed.
Results: Insulin secretion measured as the mean increase in C-peptide concentrations after one hour of hyperglycaemia were significantly lower in patients than in controls, 189 (99) pmol/l vs 1044 (433) pmol/l, $p < 0.001$. Also, the maximal C-peptide value after arginine stimulation was significantly lower in patients than in controls, 723 (583) pmol/l vs 4292 (2114) pmol/l, $p< 0.001$, This represents 38% increase in the mean C-peptide level during the first hour of hyperglycaemia in patients compared to a 97% increase in healthy volunteers, and 71% increase in patients after arginine compared to 115% in controls. Fig 1
Conclusion: There are large differences in insulin secretion between T2DM and NGT after more than seventeen years of diabetes, but rapid insulin release is still present when stimulating the b-cells with arginine. The difference in insulin response may be due to undiscovered proteins.

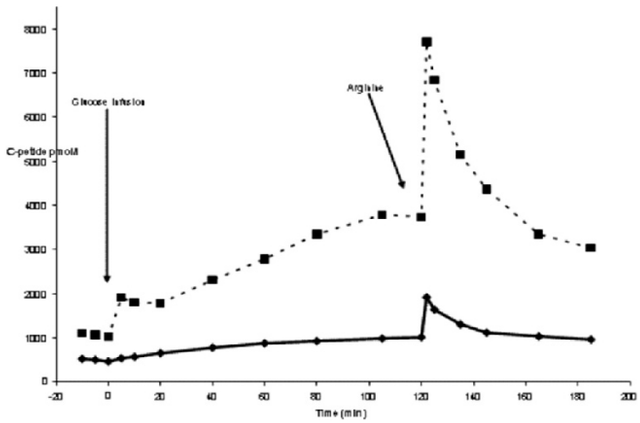

Fig 1 Mean levels of C-peptide during hyperglyceamic clamp with arginine (5g) infusion after two hours of 8 mmol/l hyperglyceamia (• and black line) and 7 healthy volunteers (dotted line)

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The aging type 2 diabetes. Differential effects of aging and diabetes on insulin sensitivity, beta cell function and incretin production

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Background and aims: Diabetic physiopathology is a combination of insulin resistance, beta cell dysfunction, and impaired incretin action or production. Aging is related to increased prevalence of T2DM. The aim of the present study is to quantitate the separate impact of aging and diabetes on the diabetic physiopathology [i.e., insulin sensitivity (IS), beta cell function and incretin production].
Materials and methods: Hyperglycemic clamp (HC) and meal tolerance test (515 Kcal) were performed in 48 subjects divided in 24 with normal glucose tolerance [NGT], and 24 with type 2 diabetes (DM) with less than 5 years of disease and taking OADs. Both NGT and DM groups were composed by 12 middle-age (35 to 50 y) and 12 aging subjects (> 65 y), with a BMI below 30 kg/m2. IS and insulin production were evaluated by HC. During the 180 minutes MTT, both incretins: GLP-1 and GIP were evaluated.
Results: The IS (Clamp-derived insulin sensitivity index - ISI, and glucose infusion rate - GIR) were reduced in DM compared with NGT ($p < 0.01$). These results were also affected by aging, but in a less intense manner when subjects were stratified by aging category ($p < 0.05$). Beta cell function (Clamp-derived derived first and second phase insulin secretion in relation to ISI - First and Second phase Disposition Indexes - DI) were reduced in DM compared to NGT ($p<0.05$). Aging did not affect DI in NGT, but exacerbates the difference between DM groups ($p<0.01$). The 180min total GIP production were similar among groups. GIP incremental area under the curve AUC(0-60 min) were greater in DM groups ($p<0.05$), but not affected by aging. The 180min GLP-1 production were reduced in aging groups ($p<0.05$), but independent of the

presence of DM. In middle age group, total GLP-1 production was reduced in DM in comparison to NGT (p<0.05). GLP-1 incremental AUC(0-60 min) were reduced in aging groups independent of the presence of DM (p<0.05).
Conclusion: In non-obese subjects, diabetic state and aging impair insulin sensitivity and incretin production independently of one another. Insulin production is affected by the DM itself, and aging exacerbates this condition. Aging associated defects superimposed diabetic physiopathology, in special regarding incretin production. The knowledge of complex relationship of aging and glucose homeostasis in diabetic and non-diabetic subjects could support the development of physiopathological-based diabetic therapies.
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Risk factors for the development of diabetes after acute beta cell mass reduction

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Background and aims: Insulin secretion (IS) defects and insulin resistance (IR) contribute to the onset and progression of type 2 diabetes (T2D), though the relative contribution of each factor is still unknown. In our series we studied the role of β cell in the pathogenesis of type 2 diabetes by analyzing patients undergoing acute β-cells mass reduction after duodeno-pancreatectomy (DP).
Methods: Eight patients were evaluated for glucose tolerance (OGTT), IS (hyperglycemic clamp), β-cell mass (arginine bolus) and insulin sensitivity (hyperinsulinemic euglycemic clamp; 40 mU/m2) before and after DP. Abdominal-CT was performed to quantify visceral adipose tissue (VAT) and subcutaneous adipose tissue volumes (SAT). During surgery we collected pancreas specimens on which we performed positive insulin area measurements (PIA) by a computer assisted system.
Results: there were 4 women and 4 men aged (mean ± SD) 58.7 ± 21.9 years, BMI (mean ± SD) 29.6 ± 6.1 kg/m2. At the enrollment no patient reported history of type 2 diabetes. Before surgery 5 patients resulted IGT. After DP, 4/8 developed diabetes, 3/8 IGT and 1/8 preserved normal glucose tolerance. There were not significative changes in whole body glucose uptake (4.3±1.1 vs. 3.8±1.1 mg/kg/min before and after surgery respectively; p=NS) following DP. A significant reduction of β-cell mass calculated as AUC of insulin after arginine bolus (AUC Arg) was detected after surgery (before 7485.8 ± 363.5 vs. after surgery 2312.2 ± 239.5 μUI/ml/min; P=0.001) as well as the I and II phase of IS (AUC I: 186.5 ± 17.4 vs. 95.0 ± 10.9 μUI/ml/min, P=0.04; AUC II: 3124.1 ± 194.3 vs. 1409.7 ± 162.4 μUI/ml/min, P=0.001). PIA was 1.67±1.43%. When AUC I and II were adjusted for beta cell mass (AUC Arg), we found a significative increase in the AUCI/AUCArg after surgery (0.05±0.03 vs. 0.14±0.12; p=0.03) but no changes in AUCII/AUCArg. We found a significant relationship of Δ(AUCI/AUCArg) with IR(r=-0,84; P=0.03) and VAT (r=0.82; P=0.03) but no with SAT, indicating that insulin resistant subjects better partially recovered the first phase of IS after surgery. In the same way PIA correlated with the degree of IR (r=-0.76;p<0.05) and VAT (r=0.9; p<0.01) but not with SAT.
Conclusion: We highlighted the unequal impact of acute β-cell mass reduction on glucose control. All patients experienced reduction in IS after DP although only half of them developed diabetes. We hypothesize that the VAT and the consequent IR may induce hypertrophy/hyperplasia of β cell mass, as documented by the strong relationship of PIA with IR and VAT. Although the IR subjects had a better partial recovery of first phase of IS, they developed diabetes. Thus, they were not able to overcome IR, indicating that loss of β-cell mass rather than the first phase plays the major role in the pathogenesis of diabetes.

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Long-term recovery of beta cell function after partial pancreatectomy in humans

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Background and aims: Glucose homeostasis and insulin secretion are significantly altered after partial pancreatectomy in humans. The present study was designed to evaluate the long-term consequences of a hemipancreatectomy in 20 patients (12 males, 8 females; age 57.4 ± 13.0 years) with chronic pancreatitis (CP; n = 10) or benign pancreatic and extra-pancreatic tumours (n = 10).
Materials and methods: In all patients, a 240 min oral glucose challenge was performed before and shortly (30.0 ± 33.9 days) after surgery, as well as after a follow-up of 3.1± 0.5 years. Plasma concentrations of glucose, insulin and C-peptide were determined, and indices of insulin sensitivity and insulin secretion were determined.
Results: In CP patients, glucose concentrations immediately after surgery were higher at t = 180-240 min compared to pre-operative levels ($p < 0.05$). At the time of follow-up, the post-challenge glucose concentrations were intermediate in between the pre-operative assessment and the experiments immediately after surgery ($p < 0.05$). Insulin secretion was reduced by ~50% immediately after surgery in CP patients ($p < 0.001$). The patterns of insulin and C-peptide concentrations determined at the time of follow-up were significantly shifted back towards pre-operative levels ($p < 0.001$), although complete normalization was still not achieved at this point in time. In the adenoma/tumour patients, fasting and post-challenge (from t = 150 - 240 min) glucose concentrations were higher immediately after surgery, but were almost completely normalized at the time of follow-up ($p < 0.001$). Likewise, post-challenge insulin and C-peptide concentrations had increased significantly compared to the early post-operative levels ($p < 0.0001$). The Matsuda index of insulin sensitivity was unchanged during the follow-up in both groups. However, the oral disposition index was restored to pre-operative levels at the time of follow-up both in CP patients (0.45 ± 0.1, 0.29 ± 0.07, and 0.60 ± 0.1 mM^{-1} , before surgery, shortly after surgery and at the follow-up, respectively; $p = 0.02$) and in the tumour/adenoma patients (1.2 ± 0.3, 0.8 ± 0.2, 1.4 ± 0.3 mM^{-1}, respectively; $p = 0.03$).
Conclusion: These findings demonstrate a capacity for recovery of glucose control after partial pancreatectomy and suggest that beta-cell function can improve significantly over time even in adult humans. Whether this is due to increases in beta-cell mass or function cannot be clarified from this study, but given the limited capacity for beta-cell regeneration in adult humans, functional improvements in insulin secretion are most likely.
Supported by: DFG

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Ghrelin suppresses insulin secretion and compromises beta cell function in healthy humans

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Background and aims: The orexigenic gut hormone ghrelin and its receptor, the growth hormone secretagogue receptor 1a, are present in pancreatic islets. While ghrelin reduces insulin secretion in rodents, its effect on insulin secretion in humans has not been established. Our objective was to test the hypothesis that circulating ghrelin suppresses glucose-stimulated insulin secretion in healthy subjects.
Materials and methods: Acyl ghrelin (0.2 and 0.6 pmol/kg/h) or saline was infused in 10 healthy subjects (4M/6F; age 29.5 ± 5.2 y; BMI 22.8 ± 3.0 kg/m^2, fasting plasma glucose 5.2 ± 0.1 pM, mean ± SEM) on 3 separate occasions in a counterbalanced fashion. The ghrelin was infused for 45 minutes to achieve steady-state levels and continued through a 180-minute frequently sampled intravenous (IV) glucose tolerance test (FSIGT). The acute (first phase) insulin response to IV glucose (AIRg) was calculated from plasma insulin concentrations between 2 and 10 min after the glucose bolus. Insulin sensitivity index (S_I) and glucose effectiveness at basal insulin (S_g) were quantified using the minimal model of glucose kinetics. Disposition index (DI), a measure of β-cell function, was a product of AIRg and S_I. IV glucose tolerance was measured by the glucose disappearance constant (Kg) from 10 to 20 min.
Results: Ghrelin infusion did not alter fasting plasma insulin or glucose, but the 0.6 pmol/kg/h dose decreased AIRg (569.6 ± 187.0 vs. 861.2 ± 288.5 min. mU/l^{-1}) and S_g (0.015 ± 0.002 vs. 0.023 ± 0.002 min^{-1}) significantly compared to the saline control ($p < 0.05$ for ghrelin vs. control). Furthermore, both the 0.2 and 0.6 pmol/kg/h ghrelin infusions decreased DI significantly (1940 ± 570, 1669 ± 628 vs. 3825 ± 1283, respectively, $p < 0.05$ for both doses vs. control). Ghrelin administration did not alter IV glucose tolerance as measured by Kg.
Conclusion: Exogenous ghrelin reduces the first-phase insulin response to IV glucose and lowers β-cell function in healthy humans. These findings raise the possibility that endogenous ghrelin has a role in control of regulation

of insulin secretion, and that ghrelin antagonists could improve β-cell function.
Supported by: NIH/NIDDK

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NEFA kinetics during an OGTT after islet transplantation
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Background and aims: In addition to its effects on carbohydrate metabolism, insulin inhibits lipolysis and promotes lipogenesis/fat storage. Abnormal NEFA kinetics are a feature of both type 1 & 2 diabetes but have not been studied in type 1 diabetes patients after intra-portal allogeneic islet transplantation. The liver is an important site of NEFA synthesis and islet recipients are known to develop periportal steatosis secondary to paracrine effects of high local concentrations of insulin. In view of the transplanted islets' heterotopic location in close proximity to hepatocytes, we aimed to evaluate NEFA dynamics in response to an oral glucose challenge in islet recipients who achieved insulin-independence.
Materials and methods: A 4-hour 75g OGTT was performed in 2 insulin-independent islet recipients, 3 patients with type 2 diabetes (T2DM) on oral medications (withheld 1 week pre-study) and 5 controls. Plasma glucose, insulin and NEFA were assayed at 0,30,60,90,120,150,180 and 240min.
Results: Mean age of islet recipients, T2DM patients and controls was 56 ± 4, 55 ± 1 and 49 ± 5 SEM years respectively; mean BMI was 19.8 ± 1.0, 37.5 ± 4.2 and 25.1 ± 1.4 SEM kg/m^2; mean HbA1c was 5.7 ± 0.3, 7.0 ± 0.3 and 5.5 ± 0.1 SEM %. Although recipients had diabetic profiles (mean glucose: fasting 6.3 ± 0.2, after 2 hours 13.0 ± 1.4 SEM mmol/l), they were insulin - independent maintaining HbA1c ≤ 6% without oral hypoglycaemics. Insulin secretion(AUCins corrected for glucose) during OGTT was lower in recipients and T2DM subjects when compared with controls(14.7%, 14.1%, 100% respectively). When corrected for prevailing insulin sensitivity, as measured by Kahn's modification of Disposition Index for OGTT[Δins(0-30min)/Δglu(0-30min)/fasting insulin], insulin secretion was 30.1% and 19.7% normal in recipients and T2DM subjects. The following table summarizes indices of NEFA kinetics in all 3 groups:

	Islet recipients (n=2)	Type 2 Diabetes (n=3)	Controls (n=5)
NEFA nadir (mmol/l)	0.014 ± 0.007	0.063 ± 0.025	0.035 ± 0.006
2 hr NEFA (mmol/l)	0.019 ± 0.010	0.130 ± 0.036	0.056 ± 0.010
Time to nadir	140 ± 20min	157 ± 19min	156 ± 6min
Time to plateau	90 ± 17min	137 ± 38min	66 ± 6min
NEFA suppression(area below basal)(0-120min) (mmol/l.min)	42 ± 11	29 ± 4	38 ± 6
% NEFA suppression to nadir †	97 ± 1	90 ± 4	93 ± 1
% NEFA suppression to 120min††	96 ± 2	80 ± 5	89 ± 2
kNEFA(30-90min) * (min^{-1})	0.039 ± 0.008	0.010 ± 0.003	0.027 ± 0.005

† (fasting NEFA - nadir NEFA)/fasting NEFA x 100%
††(fasting NEFA - 2hr NEFA)/fasting NEFA x 100%
*slope of the regression line relating the log of NEFA levels from time 30-90min
**kNEFA(30-90min)/incremental AUC_{ins} from 30-90min

Conclusion: This is the first report of NEFA suppression in intra-portal islet recipients during an OGTT. We found NEFA dynamics in islet recipients are not impaired and may be even better than in controls despite reduced peripheral insulin secretion and immunosuppressants known to cause dyslipidemia. We also found that despite marginally better glycaemia and peripheral insulin secretion when compared with T2DM subjects, recipients had disproportionately greater NEFA suppression. As the liver plays a central role in lipid metabolism/transport, high *periportal* insulin levels (secondary to proximity of transplanted β-cells to hepatocytes) in recipients could explain the normal and even improved NEFA dynamics. An alternative explanation would be that very little insulin is required for normal NEFA suppression. These preliminary findings echo those of Rickels et al, who found improved NEFA disposal in islet recipients compared with controls, during insulin-modified IVGTT. This data is of interest as improved NEFA dynamics post-transplant could contribute to enhanced glucose disposal by reducing glucolipotoxicity. Further studies, however, with larger numbers of subjects are required.
Supported by: JDRF

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Effect of ameliorating glucotoxicity on incretin secretion in patients with type 2 diabetes
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Background and aims: The condition of type 2 diabetes and accompanying hyperglycemia and insulin resistance leads to the impairment in secretion or action of the incretin hormones. In this study, we aimed to determine whether reversal of hyperglycemia and insulin resistance can affect incretin secretion in patients with type 2 diabetes. The associated factors with incretin secretion were also investigated.
Materials and methods: Meal tolerance test (MTT) was performed in eighteen poorly-controlled diabetic (pDM) patients and fifteen well-controlled diabetic (wDM) patients. Fourteen patients in pDM group underwent follow-up MTT after mean 2.4 months of insulin treatment. Plasma concentrations of glucose, insulin, C-peptide, glucagon, intact glucagon-like peptide 1 (iGLP-1) and total glucose-dependent insulinotropic polypeptide (tGIP) were measured and their secretions during MTT were calculated by total and incremental area under the curve (TAUC and IAUC) values.
Results: Post-treatment HbA1c level was significantly improved in pDM group (from 11.2 ± 0.9 to 7.9 ± 0.9%). The relative secretion of incretin hormones adjusted by glucose levels were mildly but significantly increased in pDM group after treatment (TAUCiGLP-1/TAUCglucose, from 0.07 ± 0.01 to 0.08 ± 0.01; TAUCtGIP/TAUCglucose, from 0.19 ± 0.03 to 0.24 ± 0.03; IAUCtGIP/IAUCglucose, from 0.73 ± 0.14 to 0.91 ± 0.13), although they were still significantly lower when compared to wDM group. IAUCiGLP-1 was negatively correlated with insulin resistance ($r = -0.446$, $P = 0.011$) while IAUCtGIP was positively correlated with β-cell function ($r = 0.418$, $P = 0.016$) assessed by homeostasis model assessment.
Conclusion: Intensive insulin treatment increased the relative secretory capacity of incretin hormones adjusted by glucose levels. GLP-1 secretion showed negative correlation with the index of insulin resistance. These findings indicate the importance of ameliorating glucotoxicity and lowering the degree of insulin resistance to improve incretin secretion in patients with type 2 diabetes.
Supported by: Korean Diabetes Association

PS 52 ER stress

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Acute exposure to palmitate induces endoplasmic reticulum stress and impairs insulin action in isolated human skeletal muscle strips

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Background and aims: Obesity and high fat diet have been linked to insulin resistance, which may involve endoplasmic reticulum (ER) stress. Here, we studied the effect of acute palmitate exposure on insulin action on glucose transport (GT), glycogen synthesis, insulin signaling and ER stress.
Materials and methods: We studied 18 men (47±3 years, BMI 26.2±0.8 kg/m2, fP-gluk 5.5±0.1 mM). Open muscle biopsy was obtained from m. vastus lateralis, and small muscle strips were incubated for 4 h with or without (w/o) palmitate (1mM), and with or w/o insulin (1.2 nM).
Results: Insulin increased GT (in nmol/mg/20 min) 1.9-fold (from 0.6±0.05 to 1.2 ±0.1, p<0.001). With palmitate, basal GT tended to be increased (0.9±0.1, p=0.062), insulin-stimulated GT was unchanged (1.2±0.1), and insulin action on GT (insulin-stimulated minus basal) reduced by 49 % (p=0.068). Palmitate reduced insulin-stimulated glycogen synthesis 18 % (70±13, vs 85±15 nmol/g/h, p<0.02), but did not affect AKT-Ser473 phosphorylation. When men were divided by BMI, insulin increased GT with palmitate in lean, but not in overweight men. Palmitate induced ER stress (1.3-fold increased phosphorylation of eIF2α) in overweight but not in lean men
Conclusion: Acute exposure to palmitate impairs insulin action in human muscle strips. This effect is more pronounced in men with overweight, and may involve activation of ER stress.
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ER Stress in adipocytes inhibits insulin signalling, represses lipolysis and alters the secretion of adipokines without inhibiting glucose transport

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Background and aims: ER stress and activation of the unfolded protein response (UPR) contribute to insulin resistance and the development of diabetes in obesity. It was shown previously in hepatocytes that the UPR activates c-jun N-terminal kinase (JNK) which phosphorylates insulin receptor substrate (IRS) proteins on serine residues thereby inhibiting insulin signal transduction. Here we describe how ER stress affects insulin signalling and the biological function of adipocytes.
Materials and methods: ER stress was induced with either thapsigargin or tunicamycin. Activation of insulin signal transduction and of the unfolded protein response (UPR) were assessed by Western blotting. The function of 3T3-L1 adipocytes and of primary mouse adipocytes was assessed by measuring insulin-dependent 14C-2-deoxy-D-glucose uptake, glycerol to analyse lipolysis and by multiplex analysis of culture supernatants for various cytokines.
Results: In addition to inhibition of IRS we found that ER stress downregulates the expression of the insulin receptor. Concomitantly, insulin-induced activation of Akt/PKB and of ERK1/2 was strongly inhibited. Ectopic expression of IRS1 or IRS2 strongly counteracted the inhibitory effect of ER stress on insulin signalling while pharmacological inhibition of JNK with SP600125 resulted only in a mild improvement. ER stress decreased the secretion of the adipokines adiponectin and leptin, but strongly increased secretion of IL-6. ER stress inhibited expression and insulin-induced phosphorylation of AS160, reduced lipolysis but did not inhibit glucose transport. Supernatants collected from 3T3-L1 adipocytes undergoing ER stress improved or impaired proliferation when used to condition the culture medium of INS-1E β-cells dependent on the degree of ER stress.
Conclusion: ER stress in adipocytes might initially lead to changes resembling early pre-diabetic stages which at least in part support the regulation of systemic energy homeostasis.
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Endoplasmic reticulum stress plays a role in both the adaptive and deleterious effects of lipid on insulin signalling in liver cells

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Background and aims: Insulin resistance (IR) in peripheral tissues including liver, combined with β-cell failure, leads to type 2 diabetes. Obesity and associated lipid oversupply is a likely cause of IR in liver but the mechanisms responsible remain unknown. The endoplasmic reticulum (ER) stress response has emerged as a potential signalling pathway involved in obesity-associated IR. Furthermore, high levels of fatty acids, particularly saturated forms such as palmitate, can induce ER stress in liver cells, but its necessary contribution to IR has not been elucidated. We therefore investigated whether ER stress was necessary for palmitate-induced hepatic IR. Moreover, as the ER stress response has also been described as a protective signalling pathway required for cell adaptation, we investigated the consequences of chronic mild ER stress on hepatic insulin signalling.
Materials and methods: To examine the role of ER stress in lipid-induced IR, human hepatoma HepG2 cells or mouse primary hepatocytes were exposed for 1-24 h to BSA-coupled palmitate (100-750 μmol/l) in presence or not of chemical chaperones trimethylamine N-oxide (TMAO) or tauroursodeoxycholic acid (TUDCA). To investigate the effects of prolonged mild ER stress activation on insulin signalling, HepG2 cells were exposed to low level of thapsigargin (2-2.5 nmol/l) or tunicamycin (0.1 μg/ml) for 3-6 days (two passages), or to palmitate (100-200 μmol/l) for 6-12 days (three passages). Activation of the ER stress response and insulin signalling pathway were examined using Western blot analysis and glycogen synthesis assay.
Results: In HepG2 cells, exposure to palmitate dose- (250-750 μmol/l) and time- (1-24 h) dependently led to activation of two arms of the ER stress response as evidenced by increased EIF2α phosphorylation, XBP1 splicing and JNK activation. We confirmed in hepatocytes that elevated levels of palmitate (500-750 μmol/l) induced an increase in PERK phosphorylation, XBP1 splicing and CHOP expression. In HepG2 cells and primary hepatocytes, this is associated with defective insulin signalling (decreased insulin-stimulated Akt phosphorylation) and reduced insulin action (insulin-stimulated glycogen synthesis). Treatment of HepG2 cells or primary hepatocytes with TMAO (100 mmol/l) or TUDCA (500 μg/ml), respectively, attenuated palmitate-induced ER stress and partially reversed the defect in insulin signalling, demonstrating that ER stress makes a necessary contribution to lipid-induced IR in liver cells. However, strikingly distinct effects were observed following exposure to mild ER stress. Chronic exposure of HepG2 cells to low level thapsigargin, tunicamycin or palmitate led to enhanced insulin signalling (increased insulin-stimulated Akt phosphorylation), suggesting the establishment of an ER stress adaptive pathway. This was associated with an attenuated ER stress activation in response to subsequent high-level palmitate or thapsigargin (10 nmol/l) exposure.
Conclusion: Our results suggest chronic exposure of liver cells to low-level palmitate induces mild ER stress and an adaptive response that enhances insulin signalling and confers protection against acute palmitate-induced ER stress. However, more severe ER stress induced by chronically elevated palmitate overwhelms the adaptive response leading to IR. Thus, ER stress could contribute to both the adaptive and deleterious effects of lipid on insulin signalling in liver cells.

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Endoplasmic reticulum stress induced by hyperglycaemia and saturated fatty acids is alleviated by salicylates in cultured primary human adipocytes

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Background and aims: Obesity and type 2 diabetes mellitus (T2DM) are closely associated with chronic inflammation. Adipose tissue may have a significant role in obesity associated inflammation but, the mechanisms underlying the pathogenesis of obesity induced inflammation remains unclear. Recent findings indicate that endoplasmic reticulum (ER) stress is critical to the initiation and integration of pathways of inflammation and insulin action. The ER stress occurs when there is an accumulation of unfolded/misfolded proteins, in addition to other factors. This results in activation of the

unfolded protein response (UPR) to restore functional integrity which, leads to upregulation of master regulators of ER, PKR-like ER-regulated kinase (PERK), inositol requiring enzyme1α (IRE1α) and activating transcription factor6 (ATF6) and protein chaperones. Factors acknowledged to elicit cellular stresses are hyperglycaemia, hyperlipidaemia, viral infections and increased protein synthesis; the majority of which are features of obesity and T2D. Therefore, our aims were to determine the existence and causes of ER stress in human adipocytes.
Methods: Human abdominal subcutaneous (AbSc) adipose tissue (AT) was obtained from a Caucasian non-diabetic population (BMI: 27.9±7.3 kg/m^2: age 36-49 yrs; n=40; all female subjects) that underwent elective liposuction surgery, as part of the well established AT collection program. The human preadipocytes were isolated from stromal fraction, grown and fully differentiated into human adipocytes (n=5). Well differentiated adipocytes were then treated with tunicamycin (750ng/ml), high glucose (HG) (25 mM) and saturated fatty acids (SFA) (2 mM) and in combination with 20mM salicylate, salicylate alone and controls. To characterise protein expression of the key markers relevant to ER stress, inflammation and insulin signalling pathway, total protein and RNA was extracted from adipose tissue and cultured adipocytes using standard protocol. Western blots and Real-Time RT-PCR were performed to examine protein and RNA expression levels.
Results: The expression of ER stress proteins Calnexin1, glucose regulated protein (Grp78)/BiP1, Ero-1α, protein disulfide isomerise (PDI), IRE1α and Phospho-PERK were significantly increased in AbSc AT from obese compared to lean subjects (n=4; p<0.05 to p<0.001). PERK activated phospho-eIF2α (n=5; p<0.005) was significantly induced by tunicamycin, HG and SFA (p<0.001) proving the existence of ER stress in differentiated human adipocytes. ATF6 mRNA expression was also significantly induced by tunicamycin and HG but not SFA. Grp78/Bip was also significantly induced by tunicamycin and SFA. Down-stream targets Calnexin, PDI, Ero1-Lα and Chop were also significantly induced by all three treatments (p<0.05 to p<0.001). In the same adipocytes ER stress was significantly reduced when treated with anti-inflammatory compound salicylate. Similarly, phospho-Akt (S473) (n=5; p<0.002), was also activated when ER stress was down-regulated by salicylate.
Conclusion: Our results demonstrate that hyperglycaemia (HG) and SFA induce ER stress in human adipocytes and therefore could cause increased inflammation and insulin resistance in human adipose tissue. We also demonstrate that salicylate alleviates this stress and also activates Akt which could lead to increased insulin sensitivity.
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PS 53 Metabolic surgery

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Doubling of both insulin sensitivity and beta cell function explains remission of type 2 diabetes within one week after Roux-en-Y Gastric Bypass

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Background and aims: Roux-en-Y gastric bypass (RYGB) for morbid obesity is known to cause remission of type 2 diabetes within days after surgery, but the mechanistic explanations of this phenomenon are poorly understood. Here we report the acute effects of RYGB on glucose metabolism within 4-6 days after the operation.
Materials and methods: 8 patients referred with the diagnosis of type 2 diabetes (treated with oral antiglycaemic agents or diet) and a fasting P-glucose of more than 7 mM on the last weekday before the operation, were included in the study. Patients had a BMI of 45 ± 1,8 kg/m^2, an HbA1c of 6.9 ± 0.3 and an average age of 53 ± 3 y. A mixed meal tolerance test was performed 1-3 days before and 4-6 days after bypass surgery. 3 fasting blood samples were drawn, before patients were given a 200 mL liquid meal (Fresubin Energy, 1260 kJ, protein E% 15, carbohydrate E% 50, fat E% 35), that was ingested over a 30 min period. Blood was sampled frequently over a 4 h-period after meal start for measurement of plasma glucose, insulin and c-peptide. To relate beta-cell function to the ambient insulin resistance, disposition index was calculated as the insulinogenic index (0-30 min C-peptide/0-30 min glucose) (IGI) multiplied by 1/HOMA-IR.
Results: A significant reduction in the fasting plasma glucose levels was observed after RYGB compared to preoperative levels (9.2 ± 0.98 mmol/l vs. 7.2 ± 0.43 mmol/l, p<0.05), and an even greater reduction in mean 2 h postprandial glucose levels (12.3 ± 1.2 mmol/l vs 9.2 ± 0.68 mmol/l, p<0.01) could be demonstrated. Fasting insulin levels were also reduced postoperatively (17.4 ± 3.27 μU/ml vs. 10.9 ± 2,09 μU/ml, p<0.01), as was the corresponding C-peptide levels (1.68 ± 0.21 nmol/l vs. 1.36 ± 0.24 nmol/l, p < 0.01). Accordingly, insulin resistance evaluated by HOMA-IR was reduced by almost 50% postoperatively (6.71 ± 1.12 mmol/l*μU/mL vs. 3.55 ± 0.71 mmol/l*μU/mL, p<0.01). In parallel the Matsuda Index increased from 2.6 ± 0.5 before to 4.3 ± 0.8 after surgery (p<0.05). The incremental area under the curve for C-peptide (210 ± 27 vs. 272 ± 40 min*nmol/l, p=0,055) showed a clear trend towards being greater after surgery, but failed to reach statistical significance. The insulinogenic index (IGI) was not influenced by RYGB (0.57 ± 0.10 before and 0.54 ± 0.14 after), however, the disposition index was significantly increased after compared to before surgery (0.097 ± 0.02 before vs. 0.163 ± 0.03 after, p<0,03).
Conclusion: Within one week after RYGB fasting P-glucose and 2 h postprandial glucose levels in type 2 diabetics are significantly reduced compared to pre surgical levels. These changes are associated with a near doubling of both insulin sensitivity and beta-cell function.

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Evaluation of insulin secretion and insulin sensitivity in diabetic and non-diabetic patients with morbid obesity before and after metabolic surgery

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Background and aims: In recent years metabolic surgery (MS) is widely used in patients with morbid obesity (MO) and a resolution of diabetes mellitus (DM) was seen in most patients presenting with DM before MS. The mechanisms for the "cure" of DM are still widely unknown but seem to be rather complex. Information about the change of insulin secretion and insulin sensitivity before and after MS is very limited in particular when MO obese patients with and without DM are compared.
Materials and methods: We investigated the insulin secretion and insulin sensitivity in 88 patients with MO (BMI 46.3±0.7 kg/m^2; 40.7± 1.2 years) before and 2 years after MS. Before the intervention, 16 patients had DM and 72 were nondiabetic (Non-DM). An oral glucose tolerance test (OGTT; 75 g glu-

cose) was performed before and after MS, as well as in 30 nondiabetic control (CO) subjects (mean BMI 28.4±1.4kg/m²) with similar age and sex. Insulin sensitivity was evaluated at fasting with QUICKI and during the OGTT with dynamic OGIS; ß-cell function was assessed with the insulinogenic index (IGI) as the ratio of the area under the insulin to that of glucose, representing the ability of the beta cell to respond to the glucose stimulation.
Results: The characteristics of the subjects and metabolic parameters are shown in Table 1. In the pre-operative (pre-op) state MO patients, compared to CO, showed low insulin sensitivity, both QUICKI and OGIS, and high insulin secretion (IGI). DM and non-DM only differ for the glucose pattern (p<0.003), but no difference was found for OGIS (307±55 vs. 303±19) and IGI (75±6 vs. 88±19). After surgery, OGIS and QUICKI increased till normalization (440±15 and 0.44±0.01, respectively), being similar to those of CO in all subjects (p>0.35). IGI (68±7), though markedly reduced was still higher than in CO (p=0.006). Out of the 16 MO who were diabetic pre-surgery, only 4 remained diabetic after surgery. No specific parameter, assessed pre-surgery, seems to predict the post-surgery status of the DM.
Conclusion: In conclusion, metabolic surgery, and the consequent loss of weight, is able to restore normal insulin sensitivity, even in those diabetic subjects who remain diabetic, implying that obesity per-se is more important than diabetes in modulating the metabolic parameters; i.e., when BMI is elevated, possible additional effects of diabetes are masked by those of obesity. Beta cell function remains elevated, indicating that the pancreas seems to remain up-regulated, despite the reduction of BMI and the increase in insulin sensitivity.

Table1-Patients characteristics

	all patients before MS	CO	p-value
HbA1c (%)	5.9±0.1	5.3±0.1	<0.001
BMI (kg/m²)	46.3±0.7	28.4±1.3	<0.001
QUICKI	0.36±0.00	0.46±0.01	<0.001
OGIS (ml/min¹/m²)	318.2±7.6	459.2±16.2	<0.001
Insulinogenic Index	95.1±7.1	36.8±4.1	<0.001
AUC-Glucose (mol/L 2h)	0.86±0.02	0.67±0.03	<0.001

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Improvement of first-phase insulin secretion and insulin sensitivity 72 hours after sleeve gastrectomy
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Background and aims: Obesity is a consequence of over-eating and sedentary life style. Type 2 Diabetes (T2D) is often associated and strongly related to obesity. Morbid obesity treatment actually provides medical therapy, lifestyle changes and bariatric surgery. Bariatric surgery is now considered the most successfully therapy for obesity. The purpose of this study was to evaluate the possible role of Laparoscopic Sleeve Gastrectomy (LSG) "per se" in the reversibility of type 2 diabetes immediately after surgery.
Materials and methods: We have studied insulin secretion and sensitivity in eighteen type 2 diabetic obese patients divided, by the statistical median of diabetes duration (10,5 years), in two groups: group A, patients with less than 10,5 years of disease; group B, patients with more than 10,5 years of disease. Ten non diabetic obese patients, group C, were included as control group. In all patients an Intra Venous Glucose Tolerance Test (IVGTT) was performed before and after LSG, as the following protocol: preoperatively, all patients underwent IVGTT after three days of fasting with only non caloric liquids and without antidiabetic drugs. Then, they normally fed for other three days before LSG and were submitted again to IVGTT three days after surgery without receiving neither nutrients or caloric liquids nor antidiabetic drugs, in order to avoid weight changes and interference of intestinal mechanisms on insulin secretion and sensitivity. Patients with fasting plasma glucose over 200 mg/dl before starting IVGTT were excluded.
Results: In group A, the first phase of insulin secretion promptly improved after bariatric surgery. In fact, the early insulin Area Under the Curve (AUC) significantly increased from 133.50 ± 86.70 μUI ml^{-1} · min to 254.10 ± 158.44 μUI ml^{-1} · min; p 0.012, indicating an increased glucose induced insulin secretion. The second phase of insulin secretion, expressed by the late insulin AUC, significantly decreased after SG in all groups: group A from 5275.00 ± 4533.900 μUI · ml^{-1} · min to 3552.41 ± 3326.32 μUI · ml^{-1} · min, p 0.04; group B from 3891.66 ± 2115.98 μUI · ml^{-1} · min to 1825.33 ± 988.83 μUI · ml^{-1} · min, p 0.045; group C from 15906.20 ± 3297.91 μUI · ml^{-1} · min to 3156.40 ± 2532.68 μUI · ml^{-1} · min, p 0.028. These findings suggest an improvement of insulin peripheral sensitivity.
Conclusion: Restoration of the first phase of insulin secretion, improved insulin sensitivity in type 2 diabetic obese patients, immediately after LSG, before any food passage through the gastrointestinal tract and before any weight loss, might be related to changes due to the removal of gastric fundus.

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Early and long-term effects of Roux-en-Y gastric bypass on tissue insulin-resistance in type 2 diabetic and non-diabetic morbidly obese subjects
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Background and aims: Weight loss after bariatric surgery is associated with a marked improvement of insulin resistance (IR), but the tissues involved in this effect have not been determined. Roux-en-Y gastric bypass (RYGB, a predominantly restrictive procedure) has been shown to improve IR in proportion to the weight loss in the long term; whether RYGB also has acute, weight-independent effects is controversial. Aim of our study was to measure the early and long-term effect of RYGB on IR at the level of muscle, liver and adipose tissue in morbidly obese non-diabetic and diabetic subjects.
Materials and methods: In 11 diabetic (T2D) (BMI=49±2 $kg·m^{-2}$) and 8 non-diabetic patients (OB) (BMI=55±2 $kg·m^{-2}$) matched by sex and age, we performed a euglycaemic hyperinsulinaemic clamp combined with tracers infusion (6,6-$^2H_2$-glucose and 1-^{2}H-glycerol to measure hepatic glucose production (HGP) and lipolysis (Ra-Gly), respectively) at baseline, 2 weeks and 1 year following RYGB. Muscle insulin sensitivity (M-IR = M value/steady state plasma insulin), hepatic insulin sensitivity (H-IR = HGP *x*fasting insulin) and adipose tissue insulin sensitivity (AT-IR = Ra-Gly *x* fasting insulin) were measured.
Results: Two weeks after RYGB, body weight was reduced minimally (7±1% in T2D and 4±1% in OB, p=0.01 for both); at 1 year, weight was reduced by 35±1% in T2D and 34±4% in OB (p<0.0001). Baseline fasting plasma glucose was reduced at two weeks and 1 year in both groups (7.9±0.6 *vs* 6.8±0.4 *vs* 5.2±0.2 mmol/l in T2D and 5.5±0.1 *vs* 5.3±0.1 *vs* 4.8±0.1 in OB, p<0.0001) as was fasting insulin (165±96 *vs* 92±11 *vs* 50±9 pmol/l in T2D and 125±22 *vs* 103±19 *vs* 40±5 in OB, p=0.0001). At 2 weeks, M-IS was marginally improved only in T2D (31±6 *vs* 43±6 $\mu mol \cdot min^{-1} \cdot kg_{FFM}^{-1} \cdot pM^{-1}$, p=0.05; in OB, 38±10 *vs* 36±5, p=ns), while at 1 year M-IS was doubled in OB and tripled in T2D (79±6 and 86±10 $\mu mol \cdot min^{-1} \cdot kg_{FFM}^{-1} \cdot pM^{-1}$, respectively, p<0.0001). At both 2 weeks and 1 year, H-IR was significantly reduced (2.0±0.3 *vs* 1.1±0.1 *vs* 0.7±0.1 $mmol \cdot min^{-1} \cdot kg_{FFM}^{-1} \cdot pM$ in T2D and 1.6±0.3 *vs* 1.2±0.3 *vs* 0.5±0.1 in OB, p=0.0001). At 2 weeks, AT-IR was modestly decreased only in T2D (71±12 *vs* 35±5 $\mu mol \cdot min^{-1} \cdot pM^{-1}$, p=0.003; in OB 47±11 *vs* 39±6, p=ns), while at 1 year the improvement was statistically significant in both groups (11±13 and 11±2 $\mu mol \cdot min^{-1} \cdot pM^{-1}$, respectively in OB and T2D, p<0.0001). One year after surgery, both H-IR and AT-IR were fully normalised as compared to a normal-weight control group (p=ns).
Conclusion: One year after RYGB and in concomitance with major weight loss, IR is improved in the principal target tissues (muscle, liver, adipose tissue) both in type 2 diabetic and non-diabetic subjects. In T2D patients, some improvement of tissue IR is evident acutely after surgery, in particular for liver and adipose tissue, and is likely responsible for the early amelioration of glycaemic control.

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One year effect of gastric bypass and intensive lifestyle on insulin secretion in morbidly obese patients: a controlled clinical trial
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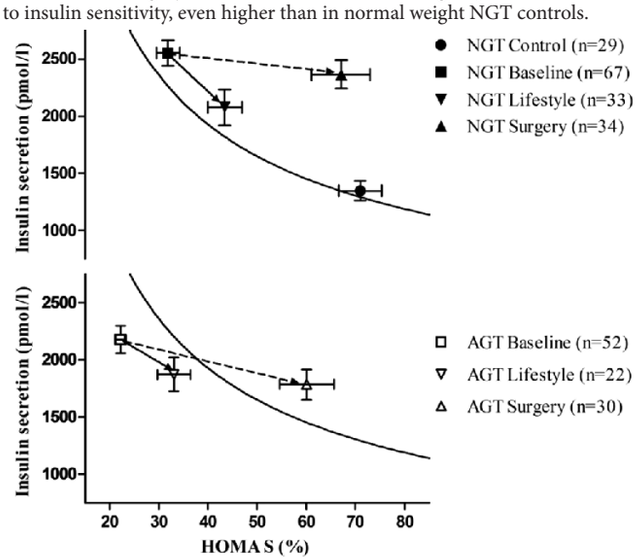
Background and aims: The effect of bariatric surgery on insulin secretion has been incompletely explained. The objective of this study was to compare the effect of bariatric surgery and lifestyle intervention on various measures of beta-cell function.

Materials and methods: The study population included 119 morbidly obese patients (mean age 44 years, mean BMI 45.5 kg/m^2, 84 women) without known diabetes classified into normal glucose tolerance (NGT, fasting glucose < 6.1 mmol/l and 2 hour glucose < 7.8 mmol/l) or abnormal glucose tolerance (AGT, fasting glucose ≥ 6.1 mmol/l and/or 2 hour glucose ≥ 7.8 mmol/l), and 29 normal weight controls with NGT. The morbidly obese subjects participated in a one year non-randomised controlled clinical trial (The MOBIL study, ClinicalTrials.gov identifier: NCT00273104) and were treated with either Roux-en-Y gastric bypass (RYGB) surgery (n=64) or intensive lifestyle intervention (ILI) at a rehabilitation centre (n=55). An oral glucose tolerance test (0, 30, and 120 min sampling) was conducted at baseline and after one year, and indices for insulin secretion (Stumvoll first phase) and insulin sensitivity (HOMA S) were calculated from glucose and insulin concentrations. Disposition index (DI), calculated as the product of Stumvoll first phase insulin secretion index and HOMA S, provided a measure of insulin secretion adjusted for insulin sensitivity. Stimulated plasma proinsulin-to-insulin ratio represented an estimate of the efficiency of proinsulin processing in beta-cells.
Results: Mean (SD) weight reduction was 30 (8) % in the RYGB group and 9 (10) % in the ILI group ($p < 0.001$). The figure shows: 1) the hyperbolic imaging of the DI in the subjects with NGT at baseline [curved line; log(HOMA S) = constant + β x log(Stumvoll fist phase) with β (95% CI) = -0.869 (-0.971 to -0.766)]; 2) higher insulin sensitivity after RYGB (dashed line) compared to ILI (solid line) (both $p \leq 0.001$) 3) a more substantial increase in the DI in the RYGB groups than in the ILI groups (both $p < 0.001$); and 4) that the DI in the RYGB NGT group was significantly higher than in the NGT control group ($p < 0.001$). The reduction in mean (SD) proinsulin-to-insulin ratio at 30 minutes was also more pronounced in the RYGB than in the ILI groups: NGT; -1.7 (1.9) % vs. 0.1 (2.1) % ($p < 0.001$); and AGT -4.3 (3.4) % vs. -1.7 (4.6) % ($p = 0.024$).
Conclusion: The improvement in beta-cell function was greater after RYGB than ILI. Post surgery, insulin secretion in the NGT group was when related to insulin sensitivity, even higher than in normal weight NGT controls.



Symbols represent mean values and error bars indicate SEM.
NGT = normal glucose tolerance. AGT = abnormal glucose tolerance.

Supported by: grants from Novo Nordisk A/S (to DH)

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Plasma FGF21 is increased in response to an oral glucose load post operative in obese subjects undergoing Roux-en-Y gastric bypass

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Background and aims: Fibroblast growth factor 21 (FGF21) is an endocrine hormone involved in regulation of energy homeostasis. Treatment of diabetic mice and diabetic rhesus monkeys with rFGF21 has been shown to lower blood glucose, correct dyslipidemia and increase energy expenditure. The physiological role of FGF21 in humans is not well understood, but FGF21 seems to be increased in states where fat oxidation is required. FGF21 has also been shown to be increased in patients with type 2 Diabetes and in obese subjects. FGF21 is highly expressed in the liver, and to a lower extent in skeletal muscle, pancreas and fat tissue. The basal level of FGF21 seems to be highly controlled by the liver, while FGF21 from the skeletal muscle is increased in response to insulin. In this study, we investigate the fasting levels of FGF21 before and after bariatric surgery, as well as the effect of an oral glucose load (25g and 50g) on plasma FGF21 and insulin before and after the surgery.
Materials and methods: 9 obese, non-diabetics patients (2 males and 7 females, average BMI 46.4; age 26 to 56) underwent Roux-en-Y Gastric Bypass. Fasting plasma FGF21 was measured on three different days before and after surgery. Furthermore, on two different days before surgery and on two different days after surgery, the subjects were given an oral glucose load (25g and 50g glucose) and blood samples were taken starting 30 min before the glucose load followed by 15 min intervals until 180 min after the oral glucose load. Statistics: Student's t-test (paired).
Results: FGF21 in plasma varied in the fasted state from 30 to 1118 pg/ml (258 ± 105 pg/ml) before surgery and from 38 to 1792 pg/ml (399 ± 170 pg/ml) after surgery. In average there was no significant change in fasting plasma FGF21. However, three subjects had a significant ($p=0.024$, $p = 0.013$ and $p=0.035$) increase in fasting plasma FGF21. Furthermore, after the surgery there was a significant ($p=0.0002$) increase in the total plasma insulin concentration during the time course, determined as the area under the curve (AUC) in response to 25g glucose, while there was no change in insulin AUC when 50g glucose was orally administered. The AUC of FGF21 was also significantly increased in response to 25g glucose ($p=0.039$), while the AUC of FGF21 in response to 50g glucose was only borderline significant ($p=0.076$). Plasma FGF21 level peaked at t=120 min and at this time point the plasma levels of FGF21 were significantly increased post surgery, in response to both 25g and 50g glucose ($p=0.015$ and $p=0.008$, respectively).
Conclusion: As previously observed, large individually variations in plasma FGF21 were found. In the nine subjects undergoing Roux-en-Y Gastric surgery, three subjects had a significant increase in fasting FGF21, while six subjects had no change in fasting plasma FGF21. The increase in plasma FGF21 in response to oral glucose post surgery is believed to arise from the stimulatory effect of insulin on the skeletal muscles and could be due to either an increase in muscle insulin sensitivity or the increased insulin release observed in response to glucose (25g). In conclusion, the increase in plasma FGF21 in response to glucose post surgery could play an important role in the positive metabolic outcome of a Roux-en-Y Gastric Bypass.

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Mechanisms of early diabetes improvement after biliopancreatic diversion in non-morbidly obese diabetic subjects

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Background and aims: A strong improvement or resolution of diabetes has been reported after biliopancreatic diversion (BPD) in diabetic obese patients. On long-term follow up, insulin sensitivity in morbidly obese subjects with or without type 2 diabetes is completely normalised after BPD despite persisting obesity. The early effect of BPD in less obese (BMI 27-35 kg/m2) diabetic subjects is not known. The aim of this study was to assess diabetes control and measure insulin sensitivity early after BPD in diabetic non-morbidly obese patients.
Materials and methods: We studied 12 patients (6 men and 6 women; 56 ± 4 years, BMI range 26.9-33.1 kg/m2) before and 59 ± 24 days after BPD (range 29-101 days). At baseline and following surgery, insulin sensitivity was measured by a 3-hour euglycaemic hyperinsulinaemic (240 pmol.m-2.min-1) clamp.
Results: After surgery, BMI decreased by 13 ± 4% (mean± SD, from 28.8 ± 1.9 to 25.0 ± 2.1 kg/m2, $p = 0.002$). Before BPD, all patients were on oral antidiabetic agents and/or insulin. After surgery, fasting plasma glucose dropped from 12.6 ± 3.1 to 8.3 ± 2.2 mmol/l ($p=0.002$) and HbA1c from 7.93 ± 1.10 to 6.42 ± 0.86%; $p=0.002$. Six patients could be taken off pharmacological treatment. Insulin sensitivity (as the M value) increased from 19.5 ± 3.4 to 34.8 ± 12.0 μmol.min-1.kgFFM-1, $p=0.002$. By simple regression analysis, the improvement in insulin sensitivity was not related to the decrease in BMI or to the time since surgery (p=ns for both).
Conclusion: In diabetic non-morbidly obese patients, biliopancreatic diversion is followed by an early improvement in glycaemic control and insulin sensitivity at a time when weight loss is modest. These findings are compatible with the notion that this type of bariatric surgery impacts on glucose homeostasis by mechanisms at least partly independent of weight loss.

PS 54 Carbohydrate metabolism

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Triple tracer (TT) and double tracer (DT) techniques are reliable methods to estimate glucose appearance in type 1 diabetes

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Background and aims: Measurement of physiological postprandial glucose fluxes in type 1 diabetes could potentially facilitate improvements in modern insulin therapy regimens. TT technique has been proposed to be the gold standard technique to measure postprandial glucose appearance. We validated TT technique and compared it against DT technique in type 1 diabetes.
Materials and methods: Eight young subjects with type 1 diabetes (age 20.8±3.3yrs, BMI 24.0±1.5kg/m2, HbA1c 8.7±1.5%, diabetes duration 10.7±8.9yrs, total daily insulin 0.8±0.2U/kg/day; mean±SD) were studied. From 1800 to 0200 next day, intravenous (iv) 20%dextrose enriched with [U-^{13}C]glucose was infused at a variable rate mimicking meal-derived glucose appearance while iv insulin was administered to achieve basal and postprandial insulin concentration. From 1530 to 0200, primed iv [6,6-$^{2}H_2$]glucose was infused in a manner that mimicked the expected endogenous glucose production. From 1800 to 0200, iv [U-^{13}C; 1,2,3,4,5,6,7-$^{2}H_7$]glucose was infused in a manner that mimicked the expected glucose appearance from a standard meal. The iv dextrose infusion was reconstructed using TT and DT techniques utilizing a modified stochastic Mari model. Plasma glucose was measured every 10-15min. Glucose enrichment was measured by gas chromatography - mass spectrometry every 10 - 30min.
Results: Figure shows actual and reconstructed dextrose infusion rates. The difference between individual actual and individual reconstructed dextrose infusion rates as assessed by the root mean square error (RMSE) was identical for the two methods (8.1± 2.1 vs. 10.6 ± 4.5 µmol/kg/min; TT vs. DT; P = NS, paired t-test). RMSE associated with mean dextrose infusion was 3.0 and 4.2 µmol/kg/min. Overall, 98 ± 9% and 93 ± 16% (P = NS) of the dextrose infusion was recovered.
Conclusion: TT and DT techniques combined with advanced computational methods can measure reliably postprandial glucose appearance in type 1 diabetes. TT tends to outperform slightly DT but the latter benefits from reduced experimental and analytical complexity.

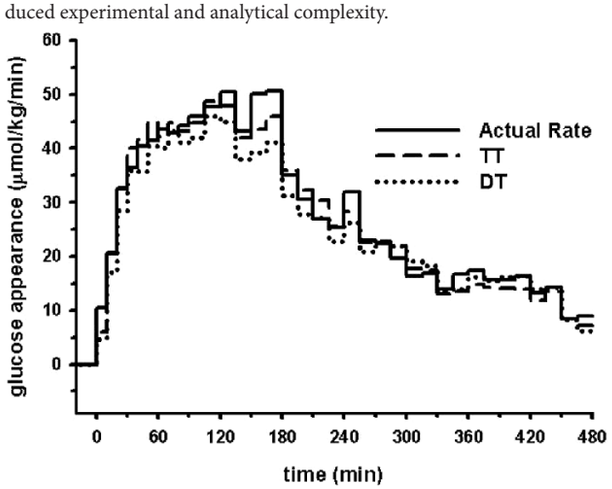

Figure. Actual infusion rate of dextrose and reconstructed infusion rate using triple tracer (TT) and double tracer (DT) techniques (N = 8, mean is shown).

Supported by: JDRF, NIHR and Diabetes UK

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Effects of impaired fasting glucose on the rate of transaldolase exchange

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Background and aims: The deuterated water ($^{2}H_2O$) method is extensively used to measure gluconeogenesis in humans. One of the premises of this method is that there is negligible exchange of the lower three carbons of fructose-6-phosphate and glyceraldehyde-3-phosphate (GAP-3-P) via transaldolase exchange. When this exchange is active, then glucose is labeled on the fifth carbon via simple exchange with labeled GAP-3-P without net gluconeogenic flux. We have recently shown that transaldolase exchange is active in healthy non-diabetic humans and ~30% of the ^{2}H on the fifth carbon of glucose is derived by this mechanism resulting in an overestimation of gluconeogenesis.
Materials and methods: To eliminate possible isotope effects associated with ^{2}H, we assessed the extent of transaldolase exchange with a ^{13}C-tracer where isotope effects are negligible. [1-^{13}C] acetate was infused in 9 subjects with impaired fasting glucose (IFG) and 11 age and BMI matched normal fasting glucose (NFG) subjects. UDP-glucose enrichment was measured following an overnight fast and during a 0.35 mu/kgFFM/min insulin infusion. Somatostatin, glucagon and growth hormone also were infused during the clamp to ensure comparable and equal portal concentrations in both groups. NMR spectroscopy was utilized to measure the ratio of [3-^{13}C] UDP-glucose and [4-^{13}C] UDP-glucose in plasma. In the absence of transaldolase exchange, carbon 4 and carbon-3 are equally labeled resulting in a C3/C4 ratio of 1.0. Transaldolase exchange selectively enriches labeling of carbon 4 resulting in a C3/C4 ratio of < 1.0.
Results: Glucose concentrations in IFG were significantly higher than NFG before (5.9 ± 0.1 vs. 5.4 ± 0.1mmol/L;p<0.005) but matched (6.3 ± 0.0 vs.6.1 ± 0.0mmol/L;p=ns) during the clamp. Insulin concentrations followed a similar trend being higher in the IFG subjects before (49 ± 5.2 vs.34 ± 3.8 pmol/L; p<0.005) but not during (109 ± 6 vs.106 ± 4) the clamp. C-peptide concentrations remained suppressed during the clamp and glucagon concentrations similar in both groups before and during clamp. The ratio of [3-^{13}C] UDP-glucose/[4-^{13}C] UDP-glucose was <1.0 in all subjects but did not differ in the IFG and NFG subjects either in the fasting state (0.68 ± 0.03 vs.0.66 ± 0.04) or during the hyperinsulinemic clamp (0.62 ± 0.04 vs.0.59 ± 0.05).
Conclusions: Transaldolse exchange a) occurs in people with NFG and IFG; b) it is not altered by an insulin infusion; and c) does not differ in NFG and IFG. Thus transaldolase exchange can account for ~35-40% of the deuterium that is present on the carbon 5 following ingestion of $^{2}H_2O$ resulting in a proportionate overestimation of gluconeogenesis. Future studies will be required to determine whether the impact of these processes on the measurement of gluconeogenesis differs in other disease states (e.g. diabetes or obesity) or changes with varying amounts of fast and/or insulin.
Supported by: NIDDK

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Protein and fat modify the glycaemic and insulinaemic responses of a mashed potato-based meal

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Background and aims: Potatoes, especially mashed potatoes, are known to result in high glycaemic and insulinaemic responses. In most meals, however, potatoes are accompanied by other foods. The objectives of the present study were to investigate how glycaemic and insulinaemic responses to mashed potato meal changed when fat, protein or/and salad were added to the meal and to assess how precise the estimate of GI of the mixed meal by using individual GI values of the components of the meal.
Materials and methods: Eleven healthy subjects (age 36.2±14.1 yrs, BMI 21.3±1.7 kg/m^2) were served the six different mashed potato-based meals (mashed potato alone; with oil; with chicken breast; with salad; with oil, chicken breast and salad; and with oil, chicken breast, salad and rye bread) containing 50-54 g of available carbohydrates once and the reference food (glucose solution) twice in a random order at one-week intervals. Capillary

blood samples were then drawn for 2 h and the glucose and insulin were analysed. The incremental areas under the curve (IAUC), glycaemic index (GI) and insulinaemic index (II) were calculated to estimate glycaemic and insulinaemic responses.
Results: The 2-hour glycaemic responses to six mashed potato-containing meals varied more than two-fold. The glycaemic index (GI) of pure mashed potato was 108, whereas combined with protein, fat and salad, it was only 54. The latter GI also differed considerably from its predicted value of 103, which was based on the individual GIs of the components of the meal. The insulinaemic indices (II) of mashed potato-based meals varied between 94 and 148. Protein in the meal increased the insulinaemic response and fat diminished it. However, the insulinaemic response to mashed potato with protein and fat was lower than for mashed potato alone.
Conclusion: The protein, fat and salad content of a meal exert considerable influence on the glycaemic and insulinaemic responses to the meal. The estimate of the GI of a mixed meal by the calculation of its components is imprecise.
Supported by: Academy of Finland, Finnish Cultural Foundation, ABS Graduate School

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Glucose appearance of large slowly-absorbed evening meal containing complex carbohydrates (CHO) in type 1 diabetes (T1D)
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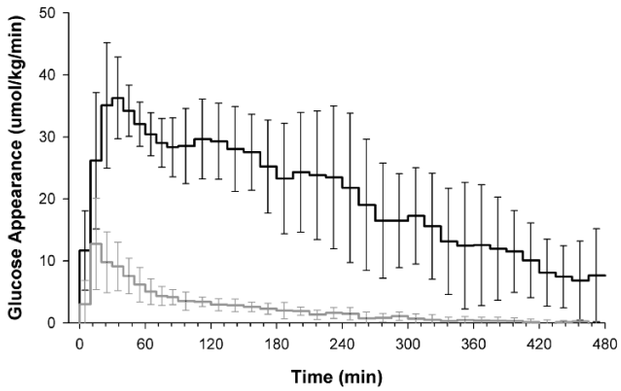
[1]University of Cambridge, [2]Addenbrooke's Hospital, Cambridge, [3]University of Surrey, Guildford, United Kingdom.

Background and aims: Many young patients with T1D report difficulty in adjusting the dose and timing of insulin when eating complex meals containing large CHO loads. Using novel methodology, we estimated appearance of complex and simple CHO in young people with T1D.
Material and methods: Eight subjects with T1D (age 20.8±3.3yrs, BMI 24.0±1.5kg/m^2, A1c 8.7±1.5%, diabetes duration 10.7±8.9yrs, total daily insulin 0.8±0.2U/kg/day; mean±SD) were studied on 2 separate visits at a clinical research facility. On both visits, from 1000 until 1730, variable intravenous (iv) insulin was infused to achieve normoglycaemia. On Visit 1 the subjects fasted until 1800 when they consumed a slowly absorbed pasta meal (CHO:protein:fat 121:35:30g; sugars:starch 13:107g; glycaemic load 54) enriched with [U-^{13}C]glucose and until 0200 next day iv insulin was infused to mimic prandial bolus of rapid-acting insulin (14±2U) and basal insulin delivery (0.9±0.3U/h). On Visit 2 identical iv insulin was given but, instead of the meal, variable iv 20%dextrose enriched with [U-^{13}C] glucose was infused to reproduce the plasma glucose profile observed on Visit 1. Iv infusion of [6,6-$^2H_2$] glucose and [U-^{13}C;1,2,3,4,5,6,6-$^2H_7$] glucose were given on both visits in a fashion to mimic endogenous glucose production (EGP) and glucose appearance of simple and complex CHO from the meal (Ra_meal), respectively. Plasma glucose enrichment with the 3 tracers was measured by gas chromatography mass spectrometry. Total glucose appearance (Ra_total) on Visit 1 was estimated by double tracer approach. Ra_meal on Visit 1 was calculated as "Ra_total - EGP", where EGP was obtained from Visit 2 and estimated by double tracer approach. Glucose appearance of simple CHO (Ra_meal_simple) on Visit 1 was estimated by triple tracer approach.
Results: Ra_meal extended over 8 hours with a sustained plateau of 30µmol/kg/min over the first 3 hours, see Figure; 25% of the Ra_meal appeared 88±21 min after meal consumption, 50% at 175±39 min, and 75% at 270±54 min. Ra_meal_simple was significantly faster with 25, 50 and 75% of the total appearance at 39±13, 84±30 and 159±42 min respectively ($p<0.0001$, paired t-test). Bioavailability of simple CHO did not differ from that of complex and simple CHO (108±25 vs 101±14%, $p=0.30$).
Conclusions: Glucose appearance after consumption of a large slowly-absorbed evening meal is sustained over 1-3hours and may extend up to 8hours. This finding may have implications on prandial insulin dosing in T1D suggesting a need for dual and/or delayed insulin doses.

Figure. Rate of appearance of complex and simple carbohydrates (black line, 120g CHO) and simple carbohydrates only (gray line, 13g CHO) from a slowly-absorbed meal [mean (SD)]



Supported by: JDRF, NIHR BRC, MRC, Diabetes UK

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Can we identify typical glycaemic patterns around meals for individuals with or without diabetes? The ADAG Study
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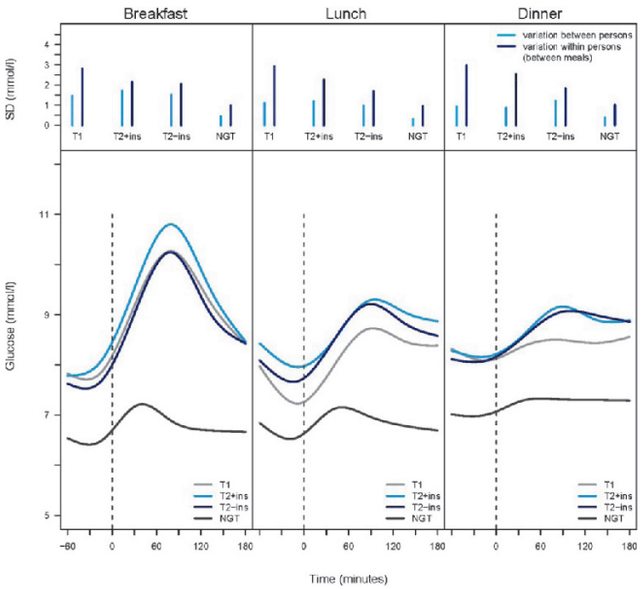
Background and aims: Postprandial glucose concentrations are determined by factors such as meal composition, physical activity, glucose tolerance, and glucose lowering therapy. Glucose profiles under real-life circumstances are not widely studied. The aim of this study was to describe the glucose profiles among persons with type 1 (T1DM), type 2 (T2DM) and in healthy control subjects (non-DM) based on intensive continuous glucose monitoring (CGM).
Materials and methods: In the A1c Derived Average Glucose (ADAG) study, participants underwent intensive glucose monitoring using CGM for approximately 13 days over a 3 month period. The study included 264 participants with T1DM, 99 with T2DM without insulin treatment, 60 with T2DM with insulin treatment, and 80 healthy controls. Multi-level mixed models with 5-point splines were fitted for 4-hour time windows around the 3 main meals of the day for each subgroup accounting for the variation between and within individuals. Mean and peak glucose levels were modelled as a linear function of age, BMI, HbA_{1c}, and the glucose reference value one hour before each meal.
Results: Glucose levels following breakfast were higher than those following lunch and dinner. Glucose concentrations peaked earlier after breakfast than after either of the other meals. The time to glucose peak value for T1DM after breakfast was 80 minutes, after lunch 95 minutes, and after dinner 100 minutes. Times to peak for T2DM without insulin treatment were 70, 85 and 100 minutes respectively. Non-DM individuals naturally presented much lower peak values, but also showed significantly shorter time to peak (50-85 minutes). The prandial glucose curves and the inter- and intra-individual variation are illustrated in the Figure.
Conclusion: Based on intensive continuous glucose monitoring in patients with T1DM, T2DM and healthy controls under real life circumstances, we found that glucose excursions after meals are largest in DM, compared to non-DM, both in terms of peak value and time to peak. The three diabetic subgroups presented similar patterns. We found considerable day to day variability in meal glucose excursions within individuals with a a stable treatment regime. This variability was larger than the variation between individuals within the same clinical subgroup. Overall, breakfast was followed by higher glucose levels than either lunch or dinner.



Bottom: Glucose levels as a function of time, age, BMI and HbA1c around the main meals of the day for the 4 clinical patient groups. The dotted line illustrates meal time.
Top: The variability (SD) of the peak glucose level within and between persons.
Supported by: ADA, EFSD, Abbott, Merck&Co, Bayer, Lifescan, GSK, Medtronic, sanofi-aventis

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Analysis of the control exerted by glucokinase and glucose-6-phosphatase on glycerol metabolism of liver cells using different systems biology frameworks

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Background and aims: The metabolic flux profile of a cell is the result of the whole of cellular processes, and is characteristic of its phenotype. Stable isotope tracer data is normally used to reveal the metabolic flux profile in cells under various conditions studied. Models of hepatic liver cells are very useful to understand the metabolic dysregulations associated to pathologies such as diabetes, due to its key role in glucose homeostasis. Glucokinase (GK) and glucose-6-phosphatase (G6Pase), the enzymes that catalyze the first futile cycle in glycolysis, have been proven to have a key role in the development of MODY2 and GSD-1 diseases. Our goal was to know if the GK-G6Pase futile cycle compromises the metabolism of glycerol, the main gluconeogenic substrate of liver cells in long fasting. The use of softwares capable of integrating all generated data allows a better extraction of relevant biological information. Thus, another aim of this study is to compare the metabolic flux profile obtained in these experiments using Mass Isotopomer Distribution Analysis (MIDA) and Isodyn, a home-made software developed for the quantification of metabolic fluxes.
Materials and methods: Primary cultures of rat hepatocytes treated with increasing doses of adenovirus encoding the rat liver GK or G6Pase, are incubated for 3h with 10mM glycerol 50% enriched in [U-13C]-glycerol. The biochemical concentrations and isotopomer patterns of glucose, lactate and glycogen are analyzed at the end of incubation. The obtained data are analyzed using MIDA and Isodyn. Using the framework of Metabolic Control Analysis, the control coefficients of GK and G6Pase over the different quantified fluxes are estimated.
Results: So far, overexpression of GK and G6Pase induced different biochemical concentrations and isotopomer patterns, which correlate with the increase of enzyme activity. The MIDA analysis of the experimental data allowed us to calculate the fluxes of glucose, glycogen and lactate synthesis from glycerol at the different enzyme activities.
Conclusion: The analysis of functional organization of metabolism based on the isotopic isomer distribution allows us to estimate the changes associated with a perturbation of the metabolic network in the central carbon metabolism. The results so far indicate that glycerol metabolism induces metabolic flux distributions that are dependent on the activity of the futile GK-G6Pase cycle. The modifications performed in our software Isodyn will make it a model valid for modelling liver cell, extracting relevant biological information and being able to integrate all generated data.
Supported by: SAF2005-01627, DIAPREPP, ETHERPATHS

PS 55 Exercise and insulin resistance

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Metabolic and anti-inflammatory benefits of eccentric endurance exercise

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Background and aims: The interplay of muscle contraction with an external force can result in one of three types of muscle activity: shortening or "concentric" when muscle contraction is stronger than the external force; lengthening or "eccentric" when the external force is stronger; and isometric when both forces are equal. Eccentric endurance exercise (e.g. hiking downwards) is less strenuous than concentric exercise (e.g. hiking upwards) but its metabolic effects are largely unknown. In the present study we therefore aimed at elucidating the metabolic effects of this training modality.
Materials and methods: We allocated 93 healthy sedentary individuals to an exercise intervention program, consisting of hiking downwards a pre-defined route in the Austrian Alps over two months. For the opposite way, a cable car was used where compliance was recorded electronically. The difference in altitude was 540 metres; the distance was covered three to five times a week. A matched group of 25 individuals served as a control group. Fasting and postprandial metabolic profiles were obtained at baseline and after the two months period.
Results: Compared with baseline, eccentric exercise significantly lowered fasting glucose (97±15 vs. 94±9mg/dl; p=0.025) and glucose tolerance (239±50 vs. 217±47 $mg^{*}dl^{-1}\ h^{-1}$; p<0.001), whereas both were unchanged in the control group (p=0.265 and p=0.231, respectively). Body mass index (27.7±4.4 vs. 27.4±4.3 kg/m^2; p=0.003) and C-reactive protein (0.27±0.42 vs. 0.23±0.25 mg/dl; p=0.031) also significantly declined in the eccentric exercise group but not in the control group (p=0.053 and p=0.864, respectively). Furthermore, eccentric exercise significantly lowered triglyceride tolerance (1959±1330 vs. 1670±1085 $mg^{*}dl^{-1}\ h^{-1}$; p=0.003) and the postprandial leukocyte count (68.8±11.6 vs. 66.5±13.6 $G^{*}L^{-1}\ h^{-1}$; p=0.031), whereas both were unchanged in the control group (p=0.819 and p=0.600, respectively).
Conclusion: Eccentric exercise is a promising new exercise modality with favourable metabolic and anti-inflammatory effects. This moderately strenuous training option could become especially important in patients with diabetes, because a large proportion of these patients suffer from comorbidities conferring a low tolerance for high-intensity training protocols.

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Comparison of Square-Wave Endurance Exercise Test (SWEET) training with endurance training targeted at the level of maximal lipid oxidation in type 2 diabetics

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Background and aims: Both low and high intensity exercise have been demonstrated to be useful in the management of type 2 diabetes. Their effects are likely to be different and complementary. We aimed at comparing in type 2 diabetes a protocol of endurance training precisely targeted at the power intensity of maximal lipid oxidation (LIPOXmax) with a protocol combining resistance and endurance training (Square-Wave Endurance Exercise Test (SWEET) training).
Materials and methods: 63 type-2 diabetics (age 52.6±1.5 years; BMI 32.7±0.7) were divided into 3 groups matched for age, BMI, and HbA_{1c} and were compared over a period of 3 months, without nutritional intervention: 39 were trained at the LIPOXmax determined with exercise calorimetry, 12 were submitted to a SWEET training, and 12 untrained patients served as controls.
Results: After 3 months, both procedures increased maximal aerobic capacity (VO2max) (SWEET training +42±16.4% p=0.027 vs LIPOXmax training (+14±4.09% p=0.0011). The effect of SWEET training on VO2max was stronger than that of LIPOXmax training (p=0.0016). SWEET training reduced resting systolic blood pressure (-12.08±5.17 mmHg p=0.040) and total cholesterol (-0.74±0.33 mmol/l p=0.049), while LIPOXmax training did not. Both procedures decreased weight and BMI. By contrast, the LIPOXmax training improved the ability to oxidize lipids (maximum lipid oxidation rate +53 ± 13.53 mg/min p=0.0005) shifted it to a higher power intensity (+20.9 ± 4.29

watts p=0.00002), decreased fat mass (- 1±0.37 %; p=0.012), increased fat-free mass (+1 ±0.37 % p=0.012), decreased waist circumference (-3.76±0.99 cm p=0.0007) and hip circumference (-2.18±0.78 cm p=0.009) while SWEET training did not significantly affect any of those parameters. Over this short period the effects of training on HbA1c were significant in the LIPOXmax group (-0.15±0.07% p=0.044) but not in the SWEET group.
Conclusion: This study shows that SWEET training improves aerobic working capacity, blood pressure and lipid profile, while low intensity endurance training (LIPOXmax training) improves the ability to oxidize lipids at exercise, increases fat free mass, decreases fat mass and decreases HbA_{1c}. The benefits of these two procedures are thus quite different and both are probably interesting to associate in the management of type 2 diabetes.

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Objectively measured sedentary behaviour and physical activity in relation to adiposity in a multi-ethnic population at risk of developing diabetes

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Background and aims: Sedentary behaviour (SB), typically measured indirectly (e.g. through self-reported TV viewing), is increasingly reported to be an important factor in the obesity epidemic, independent of physical activity. However data using objective measures of sedentary behaviour are lacking in high-risk populations. We aim to test the hypotheses that in a population identified with a high risk of diabetes, SB and light-intensity physical activity (LPA) are more important determinants of obesity than moderate-vigorous physical activity (MVPA).
Methods and materials: Participants with a high risk of diabetes, identified with the validated Leicester risk score, were recruited from primary care, Leicester, UK. SB, LPA and MVPA were measured objectively using validated accelerometers (GT3X, Actigraph), worn for 7 consecutive days. Freedson's validated cut-off points were used to calculate SB (<100 counts/min), LPA (100-1,951 counts/min) and MVPA (1,951 counts/min). Basic anthropometric measurements were conducted using standardized methods. Following best practice, total (non-sleeping) sedentary time was calculated by deducting non-wear time estimated using standardized criteria. Linear regression analysis models examined the effects of SB, LPA and MVPA on waist circumference and BMI.
Results: 89 participants were used in the analysis; mean age = 62.7 years (S.D. 9.3), Mean BMI = 33.5 kg/m^2 (S.D. 5.6), mean waist circumference = 105.5cm (S.D. 11.8). Mean daily sedentary time = 550min (S.D. 115), mean daily LPA = 248min (S.D. 73) and mean MVPA = 19min (S.D 16). After adjusting for confounders, sedentary time had the greatest effect on both waist circumference and BMI with standardised beta coefficients of 0.40 (SE = 0.18; p=0.03) and 0.31 (SE = 0.15; p=0.04) respectively (see Table 1).

Table 1 - association between different categories of activity and adiposity

	Waist circumference		**BMI**	
Sedentary time	*Standardised beta coefficient* ± S.E.	*P value*	*Standardised beta coefficient* ± S.E.	*P value*
Model 1[a]	0.57 ± 0.16	0.001	0.40 ± 0.13	0.003
Model 2[b]	0.40 ± 0.18	0.03	0.31 ± 0.15	0.038
Light intensity physical activity		P value		*P value*
Model 1[a]	-0.32 ± 0.12	0.008	-0.23 ± 0.09	0.019
Model 2[b]	-0.24 ± 1.17	0.048	-0.18 ± 0.1	0.071
moderate-to vigorous-intensity physical activity (MVPA)		P value		*P value*
Model 1[a]	-0.33 ± 0.1	0.001	-0.20 ± 0.08	0.017
Model 2[c]	-0.22 ± 0.11	0.051	-0.12 ± 0.09	0.215

[a]adjusted for wear time, gender, age, ethnic origin & smoking status
[b]adjusted for wear time, gender, age, ethnic origin, smoking status & MVPA
[c]adjusted for wear time, gender, age, ethnic origin, smoking status & sedentary time

Conclusion: Our findings conclude that in a population with high risk of diabetes, identified through a validated risk score, adiposity is more strongly linked with time engaged in SB than MVPA. Hence, diabetes prevention programmes aimed at weight loss may be more effective if the emphasis is shifted from traditional MVPA goals to reducing SB, such as sitting. If these findings are confirmed by appropriately designed intervention studies, they could have dramatic implications on future public health recommendations.

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Situation of exercise therapy for patients with diabetes mellitus in Japan - a joint project with the Japan Medical Association

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Background and aims: Regardless of the well-known health-promoting benefits of exercise, it is actually less prescribed than diet and drug therapy in the clinical practice. In addition, up till now a national survey on the present situation of exercise therapy for patients with diabetes mellitus has not been conducted in Japan. Therefore, our committee in the Japan Diabetes Society joined the Japan Medical Association to conduct the first nationwide survey that aimed to investigate the actual situation of exercise therapy for diabetic patients in Japan.
Materials and methods: Questionnaires concerning the practice and adopted systems of exercise guidance for diabetic patients were mailed to randomly selected diabetologists (n = 600) and non-specialist physicians (n = 600). Responses were obtained from a total of 403 doctors (response rate of 33.6%), 275 diabetologists (50.3 ± 10.6 years of age; mean ± SD) and 128 non-specialist physicians (52.4 ± 9.8 years of age). Collected data were analyzed using the chi-square test, the Fisher's exact test, and the Student's t-test.
Results: While only 18% of the non-specialist physicians were found to carry out special clinic for diabetic patients, this rate was 64% among diabetologists ($p < 0.001$). Diabetologists (78%) and non-specialist physicians (67%) were found to provide dietary guidance to almost all patients at the initial visit to the clinic; however, exercise guidance to the patients at their initial visit was performed by only about 40% of both categories of doctors. In addition, 10% of the diabetologists and 18% of the non-specialist physicians did not provide exercise guidance to their patients. On the other hand, while about 60% of the diabetologists provided exercise prescription and group or personal guidance to the patients, the rate among non-specialist physicians was about 30%. Less than 20% of the diabetologists answered to have physical exercise educators in their clinics. Another finding of this survey was that 46% of the diabetologists and 40% of the non-specialist physicians have no appropriate guidelines for exercise therapy of diabetic patients. Taking into consideration the main problems related with the implementation of exercise therapy by the doctors (i.e., no additional consultation fee for exercise guidance, lack of time to provide guidance, absence of specialized physical educator); it becomes clear that an early improvement of this situation is challenging.
Conclusion: The present nationwide survey on the situation of exercise therapy for diabetic patients revealed that, in Japan, (1) the proportion of dietary guidance is markedly higher than the proportion of exercise guidance and (2) significant differences between diabetologists and non-specialist physicians do exist. These results suggest that, as exercise guidelines are more realistic and effective than other demands of doctors, preparation of proper exercise guidelines for exercise therapy of diabetic patients is necessary.

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Associations between cardio-respiratory fitness and glycaemic indices in a Danish population at high diabetes risk

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Background and aims: High cardio-respiratory fitness is known to be associated with several health-outcomes such as low risk of cardiovascular diseases and all cause mortality. Furthermore, studies have shown that cardio-respira-

tory fitness is positively related to insulin sensitivity. The aim of this study was, to compare the strength of the association between cardio-respiratory fitness and multiple glycaemic indices in a population at high diabetes risk, taking into account obesity.

Materials and methods: Data from participants of the ADDITION-PRO study (cross-sectional study, subjects at high diabetes risk identified by a stepwise screening programme) are included in the analysis. Associations between fitness level and glycaemic indices are examined using linear regression models. Fitness levels were derived from estimated maximal oxygen consumption, VO_2max (mlO_2 x kg^{-1} x min^{-1}), based on the relationship between heart rate and physical activity intensity during an 8 minutes sub-maximal step test. Homeostatic model of assessment (HOMA2) was used to calculate insulin resistance (HOMA2-IR) and beta cell function (HOMA2-B). Insulin sensitivity was estimated by the Insulin Sensitivity Index (0,120). Pancreatic response was estimated by disposition index. The analyses were adjusted for sex, age, and waist circumference, WC.

Results: Data from the first 87 subjects, aged 52-77 years, 63% men, were included in the analyses. Mean VO_2max was: 32 (men) and 27 (women) mlO_2 x kg^{-1} x min^{-1}. The changes in standardized estimates of glycaemic markers per one standard deviation (SD) change in the estimated VO_2 max (partial correlation coefficients) are presented in table 1.

Conclusion: In this high diabetes risk population with low fitness levels, higher cardio-respiratory fitness was associated with better insulin sensitivity. In an age- and sex-adjusted model estimated VO_2max showed a positive association with Insulin Sensitivity Index and a negative association with HOMA2-IR reflecting the association of fitness and peripheral insulin sensitivity and hepatic insulin resistance, respectively. WC abolished these associations. However, this may be due to over-adjustment. Pancreatic response determined by disposition index and beta-cell function estimated by HOMA2-beta showed no associations with fitness level.

Table 1. Associations between estimated VO_2max and glycaemic indices

N=87	HbA1c	HOMA2-IR	HOMA2-B	Insulin sensitivity index (0,120)	Disposition index
Estimated VO_2max (mlO_2 x kg x min) adjusted for age, sex	-0.13 (-0.36;0.11)	-0.32 (-0.55;-0.10)*	-0.19 (-0.43;0.04)	0.33 (0.10;0.55)*	0.03 (-0.21;0.27)
Estimated VO_2max (mlO_2 x kg x min) adjusted for age, sex, waist circumference	-0.02 (-0.26;0.22)	-0.08 (-0.28;0.11)	-0.05 (-0.29;0.18)	0.09 (-0.10;0.29)	-0.05 (-0.30;0.20)

Partial correlation coefficients (95% CI) *p<0.01

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PS 56 Exercise: intervention

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Different responses of adipocytokine, free fatty acid, and insulin sensitivity to diet or exercise induced weight loss and maintenance in type 2 diabetes

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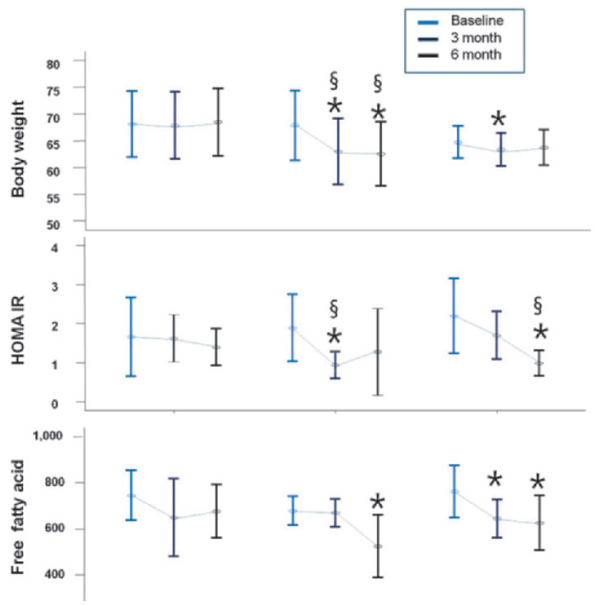
Background and aims: The aim of the study was to compare the effects of diet or exercise induced weight loss and maintenance trial on free fatty acid, adipokine, and inflammatory cytokines in type 2 diabetes.

Materials and methods: Total 39 women with type 2 diabetes were randomly assigned to control (C, N=14), diet (D, N=11), exercise (E, N=14) and completed the 3 month weight loss program, and then followed by body weight(BW) maintenance for 3 months. The restriction of calorie intake (< 1400 kcal/day) was done for D, walking for 60 minutes at moderate intensity (3.6 to 5.2 METs) five times a week for E. Diet was monitored with 3 day dietary record, and physical activity with accelerometer. We assessed anthropometric parameters, free fatty acid (FFA), high-sensitive C-reactive protein(hsCRP) and intrleukin 6(IL6) and adiponectin leptin ratio(ALr) and homeostasis model assessment of insulin resistance (HOMA- IR) at baseline, 3 months, and 6 months.

Results: At baseline, the participants' age was 54.9±7.4 years and BMI was 27.2±3.4kg/m^2 (BW: 66.8±8.7kg) without the differences across 3 groups. Body weight (BW) decreased from baseline by 4.9±1.5kg in D, and by 1.4 ±1.6 kg in E during weight loss program (p=0.001, p=0.008), and didn't change significantly during following maintenance in both intervention groups period. Increased ALr and decreased HOMA-IR were found only in D at 3 months (p=0.004, p=0.06 respectively), which didn't sustain until 6 months. HOMA-IR decreased gradually and made significant difference only at 6 months in E(P=0.04). ALr changed with BW and HOMA-IR during first 3 months(r=-0.748, p<0.001; r=-0.432, p=0.006, respectively). Within group analysis showed that FFA did not change from baseline at 3 months and decreased at 6 months in D, but decreased at 3 month and sustained until 6 month in E. FFA changed with relation to hsCRP at 3 months, and to IL6 at 6 months in all subjects((r=0.428, P=0.007: r=0.323, P=0.045, respectively).

Conclusion: These results suggest that diet induced weight loss resulted in favorable effects on insulin resistance and adipokines which were not prominent during weight maintenance. Exercise induced the improvement of insulin resistance slower than diet without relation to adipokines and body weight. On the other hand, FFA improved earlier in exercise than in diet. The lifestyle modification for 6 months was not sufficient to change the inflammatory cytokines in type 2 diabetes.



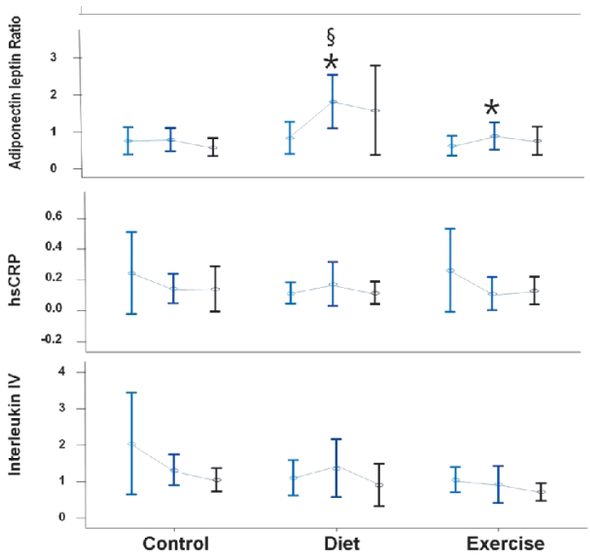


Fig. The changes of body weight, adipocytokines, and free fatty acid, and homeostasis model assessment of insulin resistance at baseline, 3 months (immediate after weight loss), and 6 months (after weight maintenance) during lifestyle modification.

§ Significant (p<0.01) compared to control group (ANOVA)
* Significant (p<0.05) from baseline (between group analysis)

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Pioglitazone increases the aerobic capacity with improved skeletal muscle energetics in patients with insulin resistance

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Background and aims: Low aerobic capacity is a strong predictor for cardiovascular morbidity and mortality in insulin resistance and type 2 diabetes. Recent study reported that pioglitazone, known as peroxisome proliferator-activated receptors (PPARs)-γ agonist, enhances the expression of genes involved in mitochondrial function and fatty acid oxidation in patients with type 2 diabetes. Skeletal muscle energy metabolism is an important determinant of aerobic capacity. Therefore, the aim of the study was to investigate the effect of pioglitazone on aerobic body (whole-body oxidative capacity) in relation to alteration in skeletal muscle energy metabolism.
Materials and methods: Fourteen male insulin-resistant subjects with no habitual exercise participated in the study before and after 3 months of 15 mg/day pioglitazone treatment. Blood was corrected in after 10 hrs fasting. To assess the aerobic capacity, peak oxygen uptake (peak VO_2) and anaerobic threshold (AT) during incremental exercise testing with cycle ergometer were measured. Body composition was measured by BOD POD, an air displacement plethysmograph and the aerobic capacity was normalized to lean body mass to eliminate the influence of altered body composition. Daily physical activity was monitored for a week before and after treatment by a pedometer with an accelerometry sensor. To assess the skeletal muscle energy metabolism, high-energy phosphates metabolites at rest and during plantar flexion exercise with a constant load of 20% one-repetition-maximum for 4 minutes were measured using ^{31}P-magnetic resonance spectroscopy (MRS). Intramyocellular lipid (IMCL) content was also measured using ^{1}H-MRS. Data are expressed as mean ± SD.
Results: Pioglitazone significantly decreased fasting blood glucose (116 ± 18 vs. 106 ± 13 mg/dL, P < 0.01), insulin (13.7 ± 9.8 vs. 6.7 ± 2.7 μIU/mL, P < 0.05), and HOMA-IR (4.0 ± 3.3 vs. 1.8 ± 0.8, P < 0.05). Body weight and %Fat were comparable before and after treatment. Daily physical activity, assessed by daily steps and movement-related calorie consumption were not altered before and after treatment. Peak VO_2 (35.1 ± 5.9 vs. 38.2 ± 5.2 mL/kg/min, P < 0.01) and AT (18.3 ± 3.0 vs. 20.2 ± 3.3 mL/kg/min, P < 0.05) normalized to lean body mass were significantly increased after treatment of pioglitazone. Phosphocreatine (PCr) loss during plantar flexion exercise was significantly decreased after treatment of pioglitazone (0.293 ± 0.112 vs. 0.256 ± 0.086, P < 0.05), suggesting increased energy reserve. Moreover, IMCL content tended to be decreased after treatment of pioglitazone (4.5 ± 1.3 vs. 3.3 ± 2.1 mmol/kg wet weight, P = 0.06).
Conclusion: Pioglitazone increased the aerobic capacity with improved skeletal muscle high-energy phosphates metabolism and decreased IMCL content in patients with insulin resistance without alteration in physical activity. These findings raise the possibility that pioglitazone might increase the aerobic capacity, at least in part, through improvement of fatty acid oxidation in skeletal muscle.

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Physical activity may offset pregnancy-related insulin resistance

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Background and aims: Insulin resistance increases in pregnancy, which is often compensated for by increase in insulin secretion in order to maintain normoglycemia. Physical activity improves insulin sensitivity outside pregnancy, but little is known of whether physical activity can offset pregnancy-related insulin resistance, which was the focus of this study.
Materials and methods: Thirty-two women in gestational weeks 28-32 from Västerbotten, Sweden were recruited through advertisement. Glucose and insulin levels were measured fasting and at 30, 60, and 120 minutes during a 75g oral glucose tolerance test. Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR). Insulin secretion was estimated via early insulin response (EIR) and the oral disposition index (DI_O). Resting metabolic rate (RMR) and total energy expenditure (TEE) were directly measured using indirect calorimetry and doubly-labeled water, respectively, from which physical activity energy expenditure (PAEE) was calculated. Heart rate measured with the Actiheart (CNT Ltd, Papworth, UK) was used to calculate time spent in moderate-to-vigorous physical activity and also to estimate sedentary time (min/day). Associations between gestational PAEE, sedentary time and insulin sensitivity or secretion were determined using generalized linear regression (SAS v9.1, Cary, NC).
Results: Total daily PAEE was positively associated with DI_O after adjustment for maternal age, height, and weight. Time spent in moderate-to-vigorous activity was not associated with any of the estimates of insulin sensitivity or secretion, but sedentary time was negatively associated with EIR.
Conclusions: Insulin resistance typically increases during pregnancy and raises the risk of gestational diabetes mellitus. These data indicate that this may be ameliorated by maintaining high levels of physical activity. Specifically, our findings suggest that minimizing time spent in sedentary behaviors might be the focus of future interventions aimed at preventing gestational diabetes mellitus.

Table 1. Tests of association between physical activity and estimates of insulinemia during pregnancy (28-32 weeks gestation)

	PAEE (kcal/day)		Sedentary time (minutes/day)		Moderate - Vigorous time (minutes/day)	
Women (n = 32)	Partial r^2	β	Partial r^2	β	Partial r^2	β
HOMA-IR[a]	0.009	-0.14	0.03	0.13	0.04	-0.19
EIR[a]	0.002	-0.06	0.15*	-0.35	0.0017	0.03
DI_O[a]	0.14*	0.38	0.008	0.19	0.03	0.13

Adjusted *p< 0.05. Multiple testing was adjusted for using the Holm procedure; Partial r^2 = variance in outcome explained by physical activity variable; β = standardized beta coefficient; PAEE = Physical activity energy expenditure; HOMA-IR = Homeostasis model assessment of insulin resistance; EIR = Early Insulin Response; DI_O = Oral disposition index; a. Corrected for maternal age, height, weight

Supported by: Foundation of: T, R Söderbergs, F, I Thurings, Västerbotten RHA

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Impact of exercise on continuously-monitored glucose levels in type 1 diabetes patients >14 years of age on insulin pump therapy

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Background and aims: Exercise is a risk factor for hypoglycemia in type 1 diabetes, but the relationship between exercise intensity and duration to the onset and severity of hypoglycemia is unclear. Here we compared the impact of standardized morning and afternoon exercise on glycemic control for 36 h post-exercise using a continuous glucose monitoring (CGM) system in type 1 diabetes patients >14 years old.
Materials and Methods: Thirty-two subjects on CGM sensor-augmented pump therapy (Paradigm REAL-Time System, Medtronic Diabetes, Northridge, CA, USA) were evaluated at the Diabetes Clinic of Hospital San Ignacio, Bogotá, Colombia. They participated in morning and afternoon exercise sessions maintaining a heart rate of 50-70% of maximum, for 4 15-min bouts of treadmill walking with 5-min breaks between the bouts. Capillary blood glucose values were measured 4 times during exercise and 8 times in the next 24 h; CGM readings were collected every 5 min in the 24 h before and 36 h after exercise. The primary outcomes was the incidence of hypoglycemia (<70 mg/dl) and hyperglycemia (>200 mg/dl) per 100 patient-hours during and after exercise. Secondary outcomes were the percentage of time in goals (70-200 mg/dl) according to the time before and after exercise and the precision of the data obtained from CGMS during exercise.
Results: Among the 32 patients exercising in the morning, there were 180 events of hypoglycemia detected by CGMS (5.6 per patient) and 39 events detected by SMBG (incidence rate 15.2 per 100 patient-hours). The highest risk of hypoglycemia was between 15 and 24 h post-exercise (ie, between 10 pm and 7 am). Of the 30 patients exercising in the afternoon, 322 events were detected by CGMS (10.7 per patient) and 62 events were detected by SMBG (incidence rate 29.0 per 100 patient-hours), and the highest risk of hypoglycemia was between 15 and 21 hours post-exercise (ie, between 7 am and 2 pm). The rates of hypoglycemia following morning and afternoon exercise were significantly different (incidence rate ratio: 0.52, 95% CI (0.43-0.63) P <0.001), but there was no between-groups difference in the risk of hyperglycemia. On the day after exercise, subjects who exercised in the morning spent 20% more time with CGM readings between 70 and 200 mg/dl (p=0.003); there was no such benefit in the afternoon exercise group, but there was an increase in percent of time with CGM readings <70 mg/dl (p=0.011). The accuracy of the data of the CGMS was evaluated by the mean absolute difference between CGM and capillary blood measurements; this was found to be 18.5% and was not affected by exercise.
Conclusions: In type 1 diabetes, exercising in the morning carries a lower risk of subsequent hypoglycemia than does equivalent afternoon exercise. The highest risk of post-exercise hypoglycaemia is between 15 and 24 hours after cessation of exercise. Morning exercise improves metabolic control on day after exercising. The use of continuous glucose monitoring during and after exercise detects a higher number of glycemic excursions than does capillary glucose measurement. CGM precision is not influenced by physical exercise.
Supported by: Medtronic, Abbott

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Examination of timing of pre-exercise administration of low GI carbohydrates on blood glucose after running in type 1 diabetes mellitus

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Background and aims: Current research recommends the use of high GI CHO immediately prior to exercise to prevent hypoglycaemia. In contrast, research examining the use of low GI (LGI) carbohydrates 2 hours before exercise demonstrates more stable blood glucose concentrations after exercise. However, given the slower digestion rates of LGI carbohydrates it is unclear if the timing of consumption of LGI carbohydrates before exercise should differ from those carbohydrates with a HGI. This study examined the blood glucose and metabolic responses to alterations in the timing of isomaltulose, a LGI carbohydrate (GI=32), before running in type 1 diabetes individuals (T1DM).
Materials and methods: Seven T1DM (31±2 years; BMI 26±0.3 kg/m^2; HbA_{1c} 8.3±0.1%) attended the laboratory on four occasions, each time consuming 75 g of isomaltulose and administering a 75% reduced insulin dose, in a randomised fashion. T1DM then remained at rest for 120 (120min), 90 (90min), 60 (60min) or 30 (30min) min before completing 45 min of running at 71±1%VO_{2peak}. Blood glucose concentrations (BG) were measured before and 3 hours after exercise. Substrate oxidation was calculated from respiratory data taken at rest and during exercise. Data (mean±SEM) were analysed using repeated measures ANOVA and expressed as changes from rest.
Results: Fasting BG was similar between conditions. Immediate pre-exercise BG under 30min (+2.8±0.2 mmol.l^{-1}) tended to be lower than 60min (+3.9±0.2 mmol.l^{-1}), 90min (+4.3±0.2 mmol.l^{-1}) and 120min (+4.1±0.6 mmol.l^{-1}), (P<0.1). BG dropped less with exercise under 30min compared to 120min (30min 4.6±0.3 vs. 120min 6.4±0.3 mmol.l^{-1}, P<0.05). In the 60 min post-exercise period BG_{auc} was different under 30min when compared to 60min and 120min (30min 7.8±0.5 *vs.* 60min 10.1±0.7, 120min 5.9±0.5 mmol.l^{-1}.hr^{-1}, P<0.05). The greatest incidence of hypoglycaemia (BG ≤3.5 mmol.l^{-1}) occurred under 120min from 60 min onwards following exercise (120min, n=5; 90min, n=2; 60min, n=1 and 30min n=0). Substrate oxidation rates during exercise displayed a strong tendency for greater lipid (30min 0.13±0.01 vs. 120min 0.07±0.01 g.min^{-1}) and lower CHO oxidation (30min 3.88±0.08 vs. 120min 4.38±0.05 g.min^{-1}) rates under 30min compared with 120min (P<0.10).
Conclusion: BG concentrations are best preserved after consumption of the low GI carbohydrate isomaltulose 30 minutes before exercise. Better preservation of post-exercise glycaemia in the 30 min ingestion trial led to a shorter digestion time before and during exercise, altering fuel metabolism towards lipid combustion and reducing the incidence of post-exercise low blood glucose.
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Heat flux is inversely associated with glucose concentrations in free living subjects with type 1 diabetes

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Background and aims: Good glycaemic control in people with type 1 diabetes is achieved by the right balance of calorie intake, exogenous insulin administration and physical activity energy expenditure (PAEE). Few trials have studied the continuous relationship between PAEE and glucose concentrations in free-living individuals during normal daily living. Most studies to date have studied fit adults in a controlled environment while following an exercise protocol. The aim of our study was to investigate the relationship between a continuous read out of glucose concentrations and daily physical activity energy expenditure in a free-living environment.
Patients and methods: 15 adults (9 female) with type 1 diabetes have been studied (age=37±10y, BMI=26.1±6kg.m^{-2}, diabetes duration=17±11y, HbA_{1c}=8.2±1.3%, mean±SD). Participants with clinical evidence of diabetic neuropathy were excluded. The participants wore a SenseWear Pro2 armband (BodyMedia Inc., USA) and a Guardian Real-Time Continuous Glucose Monitoring System (CGMS) (Medtronic MiniMed Inc., USA) continuously for up to 15 days. The armband is worn on the right triceps muscle and is only removed prior to water-based activities. The SenseWear Pro2 utilises five sensors (two accelerometers, heat flux, galvanic skin response, skin and near-body temperature sensors) to provide an estimate of overall physical activity energy expenditure. The Guardian CGMS monitors interstitial glucose concentrations from which a blood glucose value is estimated every 5mins using a proprietary algorithm. The participants were asked to perform normal daily activities and not to change their usual behaviour.
Results: Both devices were worn for 9±3days (mean±SD) and a total of 139 days of data were analysed. There was no association between mean daily PAEE, measured as area under curve for metabolic equivalent (AUC_{METs}) and mean daily glucose concentrations, measured as area under curve for blood glucose (AUC_{BG}) (r=-0.10, p=0.26), although there was a correlation between the variance in blood glucose and the variance in METs (r=0.26, p=0.002). Interestingly, there was also an association between AUC_{BG} and area under curve for heat flux (AUC_{HF}) (r=-0.26, p=0.002), even though AUC_{METs} and AUC_{HF} are strongly correlated (r=0.40, p<0.0001).
Conclusion: We show for the first time a strong inverse relationship between heat flux and a measure of glycaemic control and a positive relationship between variance in glucose concentrations and variance in physical activity levels in free living people. The data shows that greater fluctuations in physical activity are associated with greater variation in blood glucose levels throughout the day. Since heat energy (measured as heat flux) is a by-product of ATP generation in mitochondria, these data suggest that higher levels of mitochondrial activity are related to lower levels of glucose concentrations. We suggest that further work is needed to explore the relationship between ATP generation and glycaemic control and to determine whether heat flux could potentially be used as a novel non-invasive measure of glycaemic variability.
Supported by: Diabetes UK

PS 57 Glucose response *in vivo* and *in vitro*

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Glucose regulation of STIM1 translocation reflecting store-operated channel modulation in pancreatic islet cells

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Background and aims: Store-operated calcium channels (SOCs) are proposed important both for the regulation of insulin and glucagon secretion. Recent studies have identified STIM1 as a Ca^{2+} sensor in the ER. This protein oligomerizes upon Ca^{2+} store depletion and translocates to plasma membrane (PM)-adjacent regions of the ER where it interacts with the channel-forming PM molecule Orai1 that participates in the formation of SOCs. The aim of the present study was to clarify the movements of STIM1 in pancreatic islet cells under conditions known to modulate insulin and glucagon secretion.
Materials and methods: Islets isolated from C57/Bl mice were infected with adenovirus expressing STIM1 protein fused with enhanced yellow fluorescent protein (EYFP). STIM1-EYFP translocation in peripherally located islet cells was studied with confocal and total internal reflection fluorescence (TIRF) microscopy. Glucagon-releasing α- and insulin-secreting β-cells were identified by immunostaining, cell size and their different responses to adrenaline.
Results: In 3 mM glucose there was diffuse STIM1-EYFP fluorescence in peripheral islet cells with confocal microscropy, but some PM-associated fluorescence puncta were observed in TIRF microscopy. Depletion of the ER Ca^{2+} stores by inhibition of the ER Ca^{2+} ATPase with cyclopiazonic acid (CPA), which activates SOCs in α- and β-cells, induced after a delay of 118 ± 21 s pronounced formation of STIM1-EYFP puncta that preferentially associated with the PM. Less pronounced PM translocation was observed after islet exposure to Ca^{2+}-deficient medium containing EGTA. In the presence of 3 mM glucose about 70% of the islet cells reacted to 5 μM adrenaline with a loss of PM-associated STIM1-EYFP fluorescence indicating dissociation from the PM. These cells were larger than other cells and stained for insulin. In about 10% of the cells, that were smaller and stained for glucagon, adrenaline instead triggered pronounced translocation of STIM1-EYFP to the PM with formation of distinct puncta. When the glucose concentration was raised from 3 to 20 mM, there was a 248 ± 32 s delayed reduction of PM-associated STIM1-EYFP fluorescence in β-cells that only partially recovered after reintroduction of 3 mM glucose but the PM STIM1-EYFP fluorescence was rapidly restored in glucose-deficient medium. Stepwise increase of the glucose concentration from 0 to 3, 7, 11 and 20 mM induced graded reduction of PM STIM1-EYFP fluorescence with maximal effects at 3 and 11 mM in adrenaline-identified α- and β-cells, respectively.
Conclusions: The effects of CPA, glucose, and adrenaline on STIM1 localization are consistent with their stimulatory and inhibitory effects on SOCs in α- and β-cells. The glucose sensitivity of STIM1 translocation is considerably higher in α- than in β-cells, consistent with a proposed role of SOC inactivation in glucose inhibition of glucagon secretion.
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Glucose inhibits glucagon secretion from mouse pancreatic islets independently from Zn^{2+} or somatostatin, and from an action on K_{ATP} channels

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Background and aims: The mechanisms by which glucose (G) inhibits glucagon secretion are still unknown. The role of K_{ATP} channels in the G-induced suppression of glucagon secretion (GISG) is unclear and has been assessed here using NMRI mice and K_{ATP} channel modulators, or SUR1 knockout mice ($SUR1^{-/-}$), contributed by the Bryan Lab. Several hypotheses suggest that a paracrine factor, i.e. zinc released from β-cells or somatostatin released from δ-cells, is responsible for GISG. Zinc is specifically accumulated within granules of insulin by the ZnT8 transporter since no zinc exocytosis is observed in ZnT8 KO mice. In the present study, we have evaluated the role of zinc and somatostatin in GISG by using ZnT8 KO ($ZnT8^{-/-}$) and C57BL/6J mice ($ZnT8^{+/+}$, used as control), and somatostatin KO ($SST^{-/-}$) and CBA/Ca x C57BL/10 F1 mice ($SST^{+/+}$, used as control).
Materials and methods: Mouse islets were isolated by collagenase digestion of the pancreas and cultured overnight in RPMI 1640 medium containing 7 mM G. Glucagon secretion from islets was measured either in perifusion experiments in the continuous presence of a 6 mM mixture of amino-acids (2 mM alanine, 2 mM glutamine, 2 mM arginine) or in incubation experiments (only for the experiments with $ZnT8^{-/-}$ and $ZnT8^{+/+}$ islets) in the continuous presence of 20 mM arginine.
Results: Increasing the [G] of the perifusion medium from 1 to 7 mM reversibly inhibited glucagon secretion from NMRI islets. Addition of 500 μM of the K_{ATP} channel closer, tolbutamide, to a medium containing 1 mM G did not reproduce the inhibitory effect of 7 mM G, whereas the K_{ATP} channel opener diazoxide (250 μM) strongly inhibited glucagon secretion. These effects of tolbutamide or diazoxide were specific for K_{ATP} channels since they were not observed with $SUR1^{-/-}$ islets. Increasing the [G] of the perifusion medium from 1 to 7 mM in the continuous presence of 500 μM tolbutamide inhibited glucagon secretion from NMRI islets, but to a much lesser extent than in the absence of the sulfonylurea. 7 mM G also decreased glucagon secretion from $SUR1^{-/-}$ islets, but again to a lesser extent than from WT islets. In the continuous presence of 250 μM diazoxide, increasing [G] from 1 to 7 mM reversibly suppressed the already low glucagon secretion. Perifusion experiments performed with $SST^{+/+}$ and $SST^{-/-}$ islets showed that glucagon secretion elicited by 1 mM G was higher with $SST^{-/-}$ than $SST^{+/+}$ islets, and that G inhibited glucagon secretion of both types of islets. Incubation experiments performed with $ZnT8^{-/-}$ and $ZnT8^{+/+}$ islets demonstrated that G decreased glucagon secretion to the same extent in both types of islets.
Conclusion: The observations that tolbutamide did not reproduce the inhibitory effect of G, and that G could inhibit glucagon secretion in the presence of tolbutamide or diazoxide and in $SUR1^{-/-}$ islets suggest that G can modulate glucagon secretion independently from K_{ATP} channels. However, an involvement of these channels cannot entirely be ruled out because GISG was blunted by tolbutamide or in $SUR1^{-/-}$ mice. Somatostatin exerts a tonic inhibitory effect on glucagon secretion. However, neither somatostatin nor zinc are the paracrine factor responsible for GISG.
Supported by: JDRF

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Glucagon normalises disposition index by increasing acute insulin response to intravenous glucose: comparison with incretins

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Background and aims: Besides insulin resistance and defective insulin secretion, impaired glucose tolerance and type 2 diabetes are also associated with hyperglucagonemia due to inadequate suppression of glucagon production. While it is known that insulin resistance is compensated by increased insulin secretion to maintain normoglycemia, the consequence of the hyperglucagonemia as an adaptive response is not known. The model of glucose intolerance in mice given high-fat feeding is associated with markedly reduced glucose elimination after intravenous glucose, because of insufficient hyperinsulinemia. We examined whether the defective adaptations can be normalized by glucagon, as previously was documented for the incretins GIP and GLP-1.
Materials and methods: C57BL/6J mice were fed a normal chow (ND, 11%) or a diet with 60% fat for 8 weeks (HF). After baseline blood sample, a bolus injection of 35 g/kg glucose with or without addition of synthetic GIP or GLP-1 (at 3 nmol/kg) and glucagon (at 1 and 10 μg/kg) was given intravenously followed by six blood samples in 50 min with glucose and insulin measurements. The insulin sensitivity index (S_I) was estimated with the minimal model. Insulin secretion (early phase) was assessed as the increase in insulin levels within 5 min after injection (dAIR); glucose elimination (K_G) was the percent reduction of log-transformed glucose during the first 20 min; B-cell adaptation was calculated as $S_I \times$dAIR (disposition index, DI) and total B-cell sensitivity as the ratio of the area under the curve of insulin to that of glucose.
Results: Metabolic parameters are shown in Table 1. K_G was lower in HF fed mice ($P<0.0002$), normalized with incretins, but even worsened with glucagon ($P=0.008$). S_I was reduced in HF ($P<0.00001$) but neither incretins nor glucagon were able to restore a normal sensitivity. On the other hand, both incretins and glucagon yielded a marked increase of the first phase response (dAIR, $P<0.00001$): higher with glucagon, as also reflected by an elevated to-

tal B-cell sensitivity *vs.* HF and HF+incretins (P<0.02). The resulting DI was completely normalized as it was with incretins.
Conclusion: Glucagon, as the incretins, acts on the adaptive mechanisms of the B-cell by eliciting an upregulation of the response to the glucose stimulus, thus restoring a normal disposition index. This is seen in spite of being unable, as the incretins, to ameliorate insulin sensitivity in severe HF induced insulin resistant mice.

Table 1. Metabolic Parameters (means±SE)

	ND (n=89)	HF (46)	HF+incretins (28)	HF+glucagon (13)
BW (g)	22.3±0.2	35.3±1.0	38.6±1.3	30.5±0.9
K_G (%min^{-1})	1.96±0.10	1.37±0.11	2.09±0.17	0.75±0.13
S_I (min^{-1}/(pmol/l))	1.07±0.07	0.53±0.06	0.56±0.04	0.40±0.02
dAIR (pmol/l)	987±52	598±67	2207±151	2630±234
DI	11.0±0.9	3.0±0.4	12.7±1.2	10.5±0.9
B-cell sensitivity	22±1	37±3	38±3	59±5

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Preserved ability to slow gastric emptying in response to increasing concentrations of orally ingested glucose solutions in patients with type 2 diabetes

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Background and aims: The rate of gastric emptying (GE) of carbohydrate is a major determinant for postprandial plasma glucose (PG) excursions. It is well established that GE during OGTTs in healthy subjects decreases the greater the glucose amount applied. This mechanism contributes to keep postprandial PG concentrations within a narrow range averting hyperglycaemia. It is unknown whether patients with type 2 diabetes mellitus (T2DM) are able to reduce their GE rate in response to increasing orally administered glucose loads. Therefore, we aimed to investigate the GE rates in patients with T2DM ingesting 'isovolumic' glucose solutions of increasing concentrations.
Materials and methods: GE rate was measured during three 4-hour OGTTs with increasing glucose loads (25 g, 75 g and 125 g) using the paracetamol method in 8 patients with T2DM (fasting plasma glucose (FPG): 7.7 (7.0-8.9) mmol/l (mean (range)); Haemoglobin A_1c (HbA_1c): 7.0 (6.2-8.4)%) and in 8 healthy control subjects (CTRLs) matched for sex, age and BMI (FPG: 5.3 (4.8-5.7) mmol/l; HbA_1c: 5.4 (5.0-5.7)%).
Results: PG peak concentrations increased significantly among patients with T2DM with increasing glucose loads (12.8±0.5, 17.5±0.8 and 17.7±1.2 mmol/l; *p*=0.001) whereas they remained constant in CTRLs (8.9±0.5, 10.4±0.9 and 10.2±0.9 mmol/l; *p*=NS). Interestingly, equal slowing (*p*<0.05) of GE rate (as assessed by time-to-peak of plasma paracetamol) in response to increasing oral glucose loads occurred in both groups (25 g: 41±4 (T2DM) *vs.* 36±5 min (CTRL), *p*=NS; 75 g: 92±9 *vs.* 105±15 min, *p*=NS; 125 g: 131±11 *vs.* 150±16 min, *p*=NS) (Figure 1).
Conclusion: Reduced slowing of GE rate in response to increased orally ingested glucose loads does *not* seem to be a determinant for the inability of patients with T2DM to keep peak PG concentrations constant independently of the amount of glucose ingested.

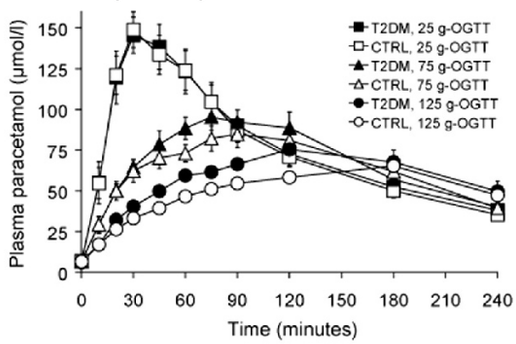

Figure 1. Plasma paracetamol concentrations during 25 g, 75 g and 125 g- OGTTs in patients with type 2 diabetes mellitus (T2DM) and healthy control subjects (CTRL).

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PS 58 Skeletal muscle, insulin action and metabolism

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Akt influences glycogen synthase in skeletal muscle through regulation of NH_2-terminal phosphorylation

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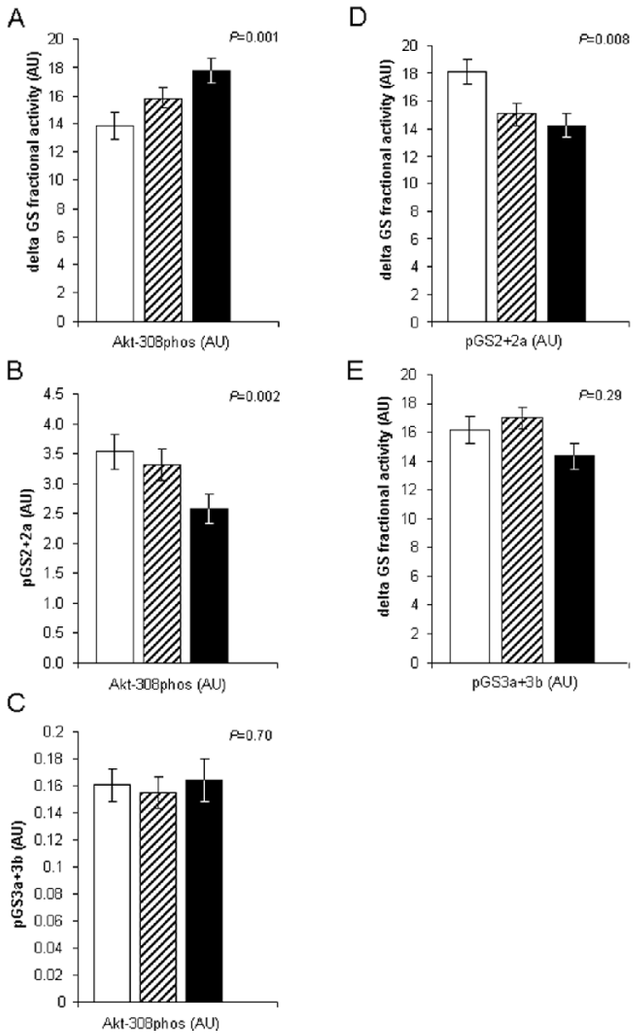
Background and aims: Type 2 diabetes is characterized by reduced non-oxidative glucose metabolism. Although studies have investigated the regulation of the key enzyme in this process, glycogen synthase (GS), the nature of the link between proximal insulin signaling and GS activation is still debated. We previously demonstrated that phosphorylation of residue threonine-308 of Akt (Akt-308phos) and Akt2 activity as well as GS activity in muscle were associated with insulin sensitivity in the present study population. Now, we aimed to evaluate the significance of each step in proximal insulin signaling on GS activity.
Materials and methods: 184 non-diabetic twins were examined with gold-standard techniques including the euglycemic-hyperinsulinemic clamp. Insulin signaling was evaluated at the levels of the insulin receptor, IRS-1-associated phosphoinositide 3-kinase (PI3K), Akt and GS employing kinase assays and phospho-specific Western blotting. Multivariate analyses adjusted for age, sex, VO_{2max}, total fat percentage and basal glycogen content were performed to evaluate the regulation of GS activity/phosphorylation status. A similar model was employed to evaluate the differences among groups in Figure 1.
Results: We demonstrated that the insulin-mediated increase (delta) of GS fractional activity was positively associated with Akt-308phos (*P*=0.002, Figure 1A) and Akt2 activity (*P*=0.008) but not Akt-473phos or insulin receptor tyrosine kinase or IRS-1-associated PI3K activity. Furthermore, Akt-308phos and Akt2 activity were negatively associated with phosphorylation of sites 2+2a (*P*<0.001 and *P*=0.001, respectively, Figure 1B) on GS which in turn was negatively associated with delta GS fractional activity (*P*=0.002, Figure 1D). We found no association between Akt and GS phosphorylation of sites 3a+3b (Figure 1C) which in turn was also not associated with delta GS fractional activity (Figure 1E).
Conclusion: Our study suggests that the association between proximal insulin signaling and GS activity in skeletal muscle primarily is mediated through Akt-dependent regulation of sites 2+2a phosphorylation of GS.

Figure 1. Delta GS fractional activity **(A)**, pGS2+2a **(B)** and pGS3a+3b **(C)** in the lowest (open bars), middle (stripped bars) and highest (black bars) tertiles of Akt-308phos and delta GS fractional activity in the lowest (open bars), middle (stripped bars) and highest (black bars) tertiles of pGS2+2a **(D)** and pGS3a+3b **(E)**. AU: Arbitrary units. Data are means±SEM.



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Coordinated myocyte expression of PPARdelta and adiponectin receptors reflects lipid metabolism, but not insulin sensitivity, of the myocyte donors

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Background and aims: Muscle lipid oxidation can be stimulated via peroxisome proliferator-activated receptor (PPAR) delta or adiponectin receptors. Both kinds of receptors sense different signals from adipose tissue: PPARdelta senses insulin resistance-associated long-chain fatty acids, adiponectin receptors the insulin sensitivity-associated adipokine adiponectin. Therefore, we asked whether myocyte expression of PPARdelta and the adiponectin receptors AdipoR1, AdipoR2, and T-cadherin reflects these different insulin sensitivity states.
Materials and methods: Skeletal myoblasts from 39 non-diabetic human donors with a broad range of insulin sensitivity were differentiated into myotubes. mRNA and 28S-rRNA contents were quantified by qPCR. Blood glucose, insulin, free fatty acids, and triglycerides were measured using standard laboratory methods. Insulin sensitivity was calculated from hyperinsulinemic-euglycemic clamp.
Results: Myocyte mRNA contents of PPARdelta, AdipoR1, AdipoR2, and T-cadherin markedly varied between the individual donors, but were not associated with the donors' insulin sensitivity (adjusted for sex, age, and BMI; all $p>0.5$). Unexpectedly, the expression levels of the four receptors were closely interrelated (all $r>=0.75$, $p<0.0001$). Furthermore, PPARdelta was, independently of the other genes, a significant determinant of T-cadherin ($p=0.0002$), but not of AdipoR1 or AdipoR2 (both $p>0.05$), suggesting T-cadherin as a PPARdelta target gene. However, GW501516 treatment did not increase T-cadherin expression ($p=0.8$, $n=16$). After adjustment for sex, age, and BMI, fasting plasma triglycerides were inversely associated with myocyte AdipoR1 and T-cadherin expression (both $p<0.03$) and tended to inversely associate with AdipopR2 and PPARdelta expression (both $p<=0.1$).
Conclusion: Human myocyte expression of PPARdelta or the adiponectin receptors AdipoR1, AdipoR2, and T-cadherin does not reflect the donors' insulin sensitivity. This lack of association might be due to these receptors' coordinated expression. Even though the physiological meaning of this integration of different pathways is currently unclear, the inverse association between myocyte receptor contents and the donors' plasma triglyceride concentrations point to its relevance for human lipid metabolism.

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TGF beta impairs muscle differentiation and induces autophagy by PED/PEA-15-mediated pathway

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Background and aims: Loss of muscle mass and de-differentiation occur in a variety of disease, including cancer, chronic heart failure, acquired immunodeficiency syndrome and diabetes. Preventing muscle wasting by promoting growth and differentiation has been proposed as a possible therapeutic approach. Tgf beta is an important negative modulator of myogenesis. However, its role in adulthood is not fully understood. Recently, it has been shown that TGF beta activates autophagy, which plays a critical role in protein breakdown, muscle atrophy and myofiber survival. Although autophagy has been found impaired in atrophying muscle, the exact mechanisms by which its deregulation might impair skeletal muscle differentiation are still unclear.
Results: Western blot and qRT-PCR analysis revealed that in L6 skeletal muscle cells, TGF beta1 stimulation increased the expression of PED/PEA-15, a gene overexpressed in type 2 diabetic patients. Moreover, Tgfbeta1 led to LC3 I/II conversion, increased expression of beclin-1 and enhanced the formation of autophagosomes in L6 cells, peculiar features of autophagy activation. These molecular events paralleled an impairment of L6 myotubes differentiation, as revealed by morphological analysis and reduction of myoD1, myogenin and glut4 gene expression. Importantly, silencing of PED/PEA-15 by specific shRNA restored the expression of myoD1, myogenin, and glut4 and L6 differentiation upon TGF beta1 stimulation. Interestingly, silencing of PED/PEA-15 also partially reverted the LC3 I/II conversion, beclin-1 levels and autophagosome formation. Furthermore, FACS analysis showed that the transfection of PED/PEA-15 cDNA in L6 cells, which we have previously described to induce insulin-resistance, activated autophagy and impaired the myotubes formation. The block of autophagy in L6PED by using 3-Methyladenine, restored myotubes formation and recovered the expression of differentiation markers. These data indicate that Tgf beta may impair skeletal muscle differentiation by inducing autophagic process and PED/PEA-15 might be a key regulator to mediate this effect.
Conclusion: These results suggest the existence of a direct relationship between insulin-resistance, autophagy and muscle de-differentiation.

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Cyclin G2: a downstream cellular marker for the mitogenicity of insulin, insulin-like growth factors and insulin analogues?

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Background and aims: The cellular and molecular mechanisms whereby some insulin analogues may cause enhanced stimulation of cell proliferation through either the insulin receptor (IR) or the IGF-I receptor (IGF-IR) are incompletely understood. Previous research in our laboratory has demonstrated differential metabolic and mitogenic signalling with some ligands in L6 rat myoblasts overexpressing the human IR (L6-hIR). While insulin was very potent in stimulating thymidine incorporation, the insulin mimetic peptide S597 had a poor mitogenic response and unlike insulin, caused a poor stimulation of the ERK/MAPK pathway. Gene expression profiling showed that the gene most downregulated (30-fold) by insulin (but 10 times less by S597)

was *ccng2*, the gene coding for cyclin G2, an atypical cyclin downstream of FOXO1 that blocks the cell cycle at the G1/S transition. Our hypothesis is that cyclin G2 may be a key downstream effector of the mitogenicity of insulin and IGFs, and therefore may be a useful cellular biomarker of mitogenicity.
Materials and methods: L6 myoblasts (WT and h-IR), were stimulated with insulin, Asp B10-insulin (X10), or insulin-like growth factor-I (IGF-I), in the absence or presence of the PI3-K inhibitors Wortmannin and LY294002 and the MAPK inhibitors PD98059 or UO126. Clinically relevant analogues were also tested. The human beta cell line INS-1 was stimulated with insulin, X10, IGF-I and GLP-1. The cells were lysed using TRI® Reagent and RNA was extracted. Gene expression was measured in a two-step qRT-PCR. The C_t values were normalized to 18S RNA and fold changes were calculated.
Results: In wild type L6 cells, expressing no IRs and 135.000 IGF-IRs, insulin was less potent than IGF-I in downregulating *ccng2* (3-fold vs 10-fold at 10 nM). Glargine, glulisine and aspart insulins had effects comparable to insulin while detemir had no significant effect on *ccng2.* In L6-hIR cells, expressing 275.000 IRs and 230.000 IGF-IRs (or hybrids), insulin, IGF-I and X10 insulin were all very potent downregulators of *ccng2*. IGF-I had the highest response, downregulating the expression of *ccng2* 50-fold after 3 hours of stimulation with 10 nM. Both insulin and X10 insulin downregulated gene expression 25-fold after 3 and 8 hours respectively. Inhibitors showed that both the MAPK and PI3-K pathways are involved in the regulation of *ccng2*. A 2 to 3-fold downregulation of *ccng2* was seen in INS-1 cells with insulin, X10 and IGF-I, as well as with GLP-1, all at 10nM.
Conclusion: X10 and IGF-I, both having a higher mitogenic potency than insulin, have a higher effect on the expression of the cell cycle inhibitor *ccng2* than insulin has, making this gene a possible candidate as a cellular biomarker for mitogenicity.

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Essential but not diabetogenic fatty acids of skeletal muscle structural and neutral lipids are associated
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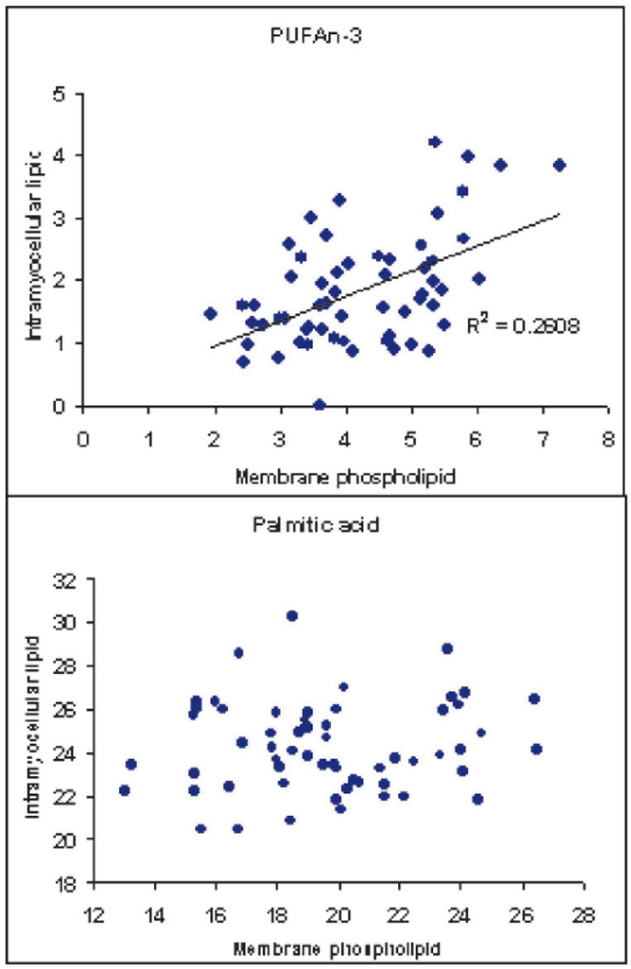
Background and aims: Fatty acid (FA) composition of structural membrane phospholipids may reflect dietary FA sources in man. However, it is unknown the extent to which skeletal muscle phospholipid FA composition reflects and/or is associated with intramyocellular triglyceride FA composition. In particular, the association between structural and intramyocellular lipids for essential FA of marine origin facilitating insulin action and the saturated FA palmitic acid, respectively, is of interest to study due to their different effects on health including insulin action.
Materials and methods: Vastus lateralis skeletal muscle biopsies were obtained from 59 weight-stable sedentary subjects, i.e. 27 obese women (7 diabetic), 20 obese men (6 diabetic), and 12 lean healthy normal-weight subjects (7 women). FA composition of neutral and structural fat was determined by thin-layer and gas-liquid chromatography.
Results: In structural versus intramyocellular lipids, the concentration of essential FA of dietary origin including linoleic (C18:2n-6) and linolenic (C18:3n-3) FA correlated significantly (r=0.34, P<0.01; r=0.62, P<0.001). Similarly, the concentration of polyunsaturated FA of marine origin (r=0.51, P<0.001) including the ratio of n-3/n-6 (r=0.56, p<0.001) polyunsaturated FA was also significantly correlated between structural and intramyocellular lipids. The correlations remained virtually unaffected after correction for gender, percentage of body fat mass and insulin resistance (partial correlations, r=0.32; r=0.66; r=0.51; r=0.56, respectively). Total monounsaturated FA (r=0.43, P<0.001; r=0.36, P<0.01)) and trans FA (r=0.33, P=0.01; r=0.32, P<0.02) correlated between the two types of lipids using both univariate as well as corrected analyses. In contrast, total saturated fat (r=0.01, P=ns), including the potentially "diabetogenic" palmitic acid (C16:0, r=0.10, P=ns), did not correlate between structural and intramyocellular FA composition.
Conclusion: Dietary essential FA ingestion may influence the intramyocellular lipid composition. However, the levels of saturated FA including palmitic acid may differ substantially between structural phospholipids versus intramyocellular lipids. Understanding the underlying differential mechanisms regulating structural versus intramyocellular fat metabolism may have implications for health including insulin action.

Figure
The associations of PUFAn-3 and palmitic acid in skeletal muscle membrane phospholipids vs. intramyocellular lipids



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Suppression of the 5´nucleotidase NT5C2 promotes AMPK phosphorylation and lipid oxidation in human skeletal muscle
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Objective: The 5'Nucleotidases (NT5) family of enzymes dephosphorylate non-cyclic nucleoside monophosphates to produce nucleosides and inorganic phosphates. We hypothesized that gene silencing of 5'Nucleotidases to increase the intracellular availability of AMP would increase AMPK activity and metabolism.
Research design and methods: The present study was designed to determine the role of the cytosolic 5' nucleotidases in the metabolic responses liked to the development of insulin resistance in obesity and type 2 diabetes.
Results: mRNA expression of NT5C1A and NT5C2 were elevated in extensor digitorum longus (EDL) and soleus muscle of *ob/ob* mice compared to wild-type litter mates, respectively. NT5C1A mRNA was increased in vastus lateralis skeletal muscle obtained from type 2 diabetic patients, compared to normal glucose tolerant subjects. Using siRNA to silence NT5C2 expression in cultured human myotubes, we observed a 2-fold increase in AMPK phosphorylation (Thr^{172}) and a 4-fold increase in phosphorylation of its downstream target ACC (Ser^{79}) (p<0.05). siRNA silencing of NT5C2 expression increased palmitate oxidation 2-fold in the absence and 8-fold in the presence of AICAR. This response was paralleled by an increase in glucose transport

and a decrease in glucose oxidation and incorporation into glycogen and lactate release from NT5C2 depleted myotubes.
Conclusion: Our results provide evidence to suggest that endogenous NT5C2 inhibits basal lipid oxidation and glucose transport in skeletal muscle. Reduction of 5'Nucleotidase expression or activity may be one potential avenue to promote metabolic flexibility in type 2 diabetes.
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Bombesin receptor subtype-3 signalling and its role on glucose transport in human myocytes
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Background and aims: Bombesin Receptor Subtype-3 (BRS-3) is one of the candidate genes of obesity; in fact, BRS-3-knock-out mice is characterized by hypertension, insulin resistance, mild obesity, impaired GLUT-4 translocation in adipocytes and unbalanced glucose metabolism, suggesting a role of BRS-3 in glucose homeostasis. Previously, it was shown not only that human skeletal muscle expresses functional BRS-3, but also a lower than normal levels of its gene expression in muscle tissue from Type-1, Type-2 and obese patients. The aim of this study was to gain insight into the BRS-3 signalling pathways in normal human myocytes, by using the BRS-3 agonist peptide [D-Tyr(6),βAla(11),Phe(13),Nle(14)]Bn-(6-14)] (BRS-3-AP), and to explore the action of BRS-3-AP upon glucose transport (GT).
Materials and methods: Primary culture myocytes were established from skeletal muscle pieces (≈400 mg), obtained, previous informed consent given, from five normal subjects (5F; age: 49±9 yr; fasting plasma glucose: 96±6 mg/dl) undergoing surgery. PKB and p42/44 MAPKs activity - phosphorylation degree- was measured by immunoblotting, in cells after 3 min incubation in the absence (control) and presence of BRS-3-AP (10^{-10}-10^{-8} M); GT was examined as ^{3}H-2-deoxy-D-glucose incorporation, in the absence and presence BRS-3-AP (10^{-12}-10^{-7} M) and without and with 10^{-6} M wortmannin or $2.5x10^{-5}$ M PD-98059, respective inhibitors of PI3K/PKB and MAPKs activity; insulin was also included in all assays as positive control.
Results: BRS-3-AP, at 10^{-9} M and 10^{-8} M, clearly increased (p<0.05) PKB phosphorylation (168±17% control and 192±42% control, respectively) to the same level as that reached by 10^{-9} M insulin (175±28%, p<0.05); however, while at 10^{-10} M and 10^{-9} M BRS-3-AP failed to modify the global MAP kinases activity (overall mean value: 101±7% control), a clear increase (p<0.05) in the phosphorylation level of p42/44 MAPKs occurred when 10^{-8} M BRS-3-AP was present (p-p42: 160±22% control; p-p44: 142±14%), values of the same of magnitude as those by 10^{-9} M insulin (p-p42: 131±2% control; p-p44: 126±9%). BRS-3-AP caused a concentration-related stimulation of GT, which was already detected at 10^{-10} M of the peptide (133±11% control, p<0.05), maximal at 10^{-9} M (159±13%, p<0.01) and maintained thereafter up to 10^{-7} M BRS-3-AP (10^{-8} M: 158±18% control, p<0.02; 10^{-7} M: 196±36, p<0.05), effect which was similar to that induced by insulin. Wortmannin abolished the stimulatory action of 10^{-8} M BRS-3-AP on GT (102±7% control), and the same blocking effect was observed in the additional presence of PD-98059 (94±4%).
Conclusion: These results implicate human BRS-3 in the glucose homeostasis process, and open the possibility that this receptor could be used as a molecular target, and/or its agonist peptide, in the therapy of diabetes and obesity.
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The role of phospholemman in the regulation of glucose uptake in insulin sensitive tissues
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Background and aims: Phospholemman (PLM, FXYD1) is a phosphoprotein expressed in the cell plasma membrane in different tissues including adipose tissue, liver, heart and skeletal muscle. Phosphorylation of PLM leads to an increase in Na^+/K^+ pump activity and thus regulates active ion transport. In adipocytes PLM is reported to be involved in insulin-induced GLUT4 translocation to the plasma membrane. The aim of this study was to determine the role of PLM in insulin-induced glucose uptake in skeletal muscle and adipocytes.
Materials and methods: L6 myoblasts, which have a intrinsically low PLM expression, were transfected with plasmids encoding either wild-type PLM, phosphorylation-mutant Ser68Ala-PLM or double-mutant Ser63Ala/Ser-68Ala-PLM. Thereafter, insulin-stimulated glucose uptake was measured. Interaction of GLUT4 and PLM was assessed by co-immunoprecipitation. 3T3-L1 fibroblasts were induced to differentiation into adipocytes. In differentiated adipocytes, siRNA PLM silencing was performed and insulin-stimulated glucose uptake was measured.
Results: PLM overexpression increased insulin-stimulated glucose uptake in comparison with mock transfected cells, whereas overexpression of mutant Ser63 and/or Ser68 had no effect on insulin-stimulated glucose uptake. Basal glucose uptake was unaltered by overexpression of PLM. PLM co-immunoprecipitates with GLUT4 in rat epitochlearis muscle and this interaction is increased after insulin stimulation. In 3T3-L1 cells the induction of PLM expression follows GLUT4 expression during differentiation into adipocytes. The silencing of PLM in 3T3-L1 adipocytes leads to decrease in insulin-stimulated glucose uptake.
Conclusion: PLM over-expression enhances insulin-stimulated glucose uptake in L6 myoblasts, and the effect is dependant upon PLM phosphorylation on Ser63 and/or Ser68 residues. Increased co-immunoprecipitation of PLM and GLUT4 after insulin stimulation provides evidence to suggest that an interaction between PLM and GLUT4 may be involved in regulation of the glucose uptake. Furthermore, the expression level and phosphorylation status of PLM appears to be important for the regulation of insulin-stimulated glucose uptake in insulin sensitive tissues.
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The beneficial effect of electro-acupuncture on insulin resistance
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Background and aims: Electro-acupuncture (EA) improves insulin resistance, although the biochemical mechanism underlying this effect remains unclear. This study investigated the effect of low-frequency EA on metabolic action in genetically insulin-resistant diabetic db/db mice.
Materials and methods: Nine-week-old db/m and db/db mice were randomly divided into four groups: db/m, db/m+EA, db/db, and db/db+EA. Db/m+EA and db/db+EA mice received 3-Hz EA five times/wk for 8 weeks.
Results: The EA reduced fasting blood glucose and maintained insulin levels without significant alteration of food intake or body weight in db/db mice. Improved insulin sensitivity was established in EA-treated db/db mice by intraperitoneal insulin tolerance test. EA also decreased free fatty acid levels in the db/db mice and increased skeletal muscle sirtuin 1 (SIRT1) protein expression. These effects induced concurrent upregulation of the genes related to mitochondrial biogenesis such as peroxisome proliferator-activated receptorγ (PPARγ) coactivator 1alpha (PGC-1alpha) and nuclear respiratory factor 1 (NRF1). Furthermore, EA treatment activated AMP-activated protein kinase (AMPK) and increased Akt phosphorylation in skeletal muscle of the db/db mice.
Conclusion: These findings suggest that EA has a beneficial effect on insulin resistance, at least partly via stimulating SIRT1/PGC-1alpha and AMPK activity, resulting in improved insulin signal defect.

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A mRNA marker for glycolytic muscle fibres may be used to determine fibre type composition in human skeletal muscle
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Background and aims: Human skeletal muscle contains three major fibre types distinguished by their myosin heavy chain (MHC) isoforms. The mitochondria content and oxidative capacity is the highest in slow oxidative type I fibres, lower in fast oxidative type IIa fibres, and lowest in fast glycolytic type IIx/d fibres. The amount of oxidative type I fibres is reduced and

glycolytic type IIx/d fibres is increased in muscle from patients with type 2 diabetes. We have previously shown that mRNA expression level of markers for oxidative fibres were positively related to insulin-stimulated glucose uptake and VO2max, meanwhile mRNA expression level of markers for glycolytic fibres were negatively related to these metabolic factors. The aim of the present study was to investigate whether mRNA expression levels of fibre type markers can be used as a proxy to determine fibre type composition in human skeletal muscle.
Materials and methods: Human skeletal muscle biopsies were analysed from two different cohorts (cohort 1 n=36 and cohort 2 n=37). An ATPase staining method was used to determine the fibre types. The expression levels of three fibre type markers; *MHC7* (slow oxidative), *MHCIIa* (fast oxidative) and *MHCIIx/d* (fast glycolytic), were measured using microarrays and the mRNA expression was correlated with muscle fibre type.
Results: By using bivariate non-parametric correlation tests, we found that *MHCIIx/d* mRNA expression was positively correlated to the level of type IIx/d muscle fibres in both cohorts (cohort 1: r=0.39 and p=0.020; cohort 2: r=0.59 and p=0.00023), meanwhile mRNA expression of *MHC7* and *MHCIIa* did not significant correlate with levels of its respective fibre type in neither cohort. When using regression analysis corrected for BMI, age and diabetes status, the same results were obtained. *MHCIIx/d* mRNA expression was positively related to type IIx/d fibres in both cohorts (cohort 1: beta = 0.04 ± 0.002 and p=0.016; cohort 2: beta= 7.99 ± 2.92 and p=0.020), meanwhile no significant relationship was found between *MHC7* mRNA expression and amount of type I fibres or between *MHCIIa* mRNA expression and amount of type IIa fibres in neither cohort. Interestingly, mRNA expression of *MHCIIx/d* was negatively correlated to both amount of type I and type IIa fibres in cohort 2 (r= -0.41 and p=0.012, respectively, r= -0.38 and p=0.028).
Conclusion: Fibre type composition has been shown to be of importance in the pathogenesis of type 2 diabetes and a practical marker is needed. Here we show that the mRNA expression level of *MHCIIx/d*, may be used as such a marker for fast glycolytic muscle fibres in human skeletal muscle.

PS 59 Insulin action and metabolism in adipose cells

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Microfluidic technology for multi-parametric studies on patient-derived three-dimensional human adipose tissue model
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Background and aims: Type 2 Diabetes Mellitus is a complex disease affecting many pathways in different tissues. The complexity of this disease led to the use of several classes of drugs acting with different mechanisms and targets and with effects which often change between patients. The screening of all these anti-diabetic drugs with animal models is not economically and timing sustainable and often not giving reliable results for human. On the other hand, specific study on human patients are possible but are tremendously expensive and require a huge effort in term of ethical approval and safety issues. Within this scenario we aim at developing a microfluidic platform allowing to perform in vitro highthroughput patient-specific tests of anti-diabetic drugs on patient-derived three-dimensional human adipose tissue. In particular, the first step is the realization of a microfluidic system for culturing human adipose tissue able to control the temporal evolution of culture conditions in terms of concentration of oxygen, metabolites, and insulin and able to perform multi-parametric analyses of the adipose tissue behaviour.
Materials and methods: Biopsies of subcutaneous and visceral adipose tissues were obtained from both patients affected by Type 2 Diabetes and insulin-sensitive individuals. 1cm^3 biopsy was minced right after surgery into 10-20mg tissues. Each piece was placed in a 24well plate with 1ml medium for 24h. Then the tissue was either cultured for additional time in the 24well plate with fresh medium or placed into the microfluidic system. A microfluidic platform including micro-valves, injectors, pumps, mixers was realized by soft-lithographic technique and its design, development, and application was assisted by mathematical modeling. In line measurements of tissue metabolic activity were performed using micro-biosensors placed downstream the culture chambers and able to detect glucose, lactate and oxygen concentration. The tissue responses to insulin were investigated also through analyses of free fatty acids and glycerol. Viability and histological analyses were performed at the end of the cultures.
Results: Microscale adipose tissues were cultured within the microfluidic platform for up to 4 days. MTT assay at the end of the culture showed high tissue viability and no significant differences with controls in 24well plates. On the other end, the microfluidic system allowed a two times higher glucose uptake then the controls by reducing the glucose diffusive resistance. We then investigated the effect of different insulin concentrations (20, 40 and 100nM). Preliminary results obtained with tissues of insulin-sensitive individuals showed an high variability between biopsies and between cultures from the same biopsy. However, we observed an enhancement of glucose uptake for increasing insulin concentration when using 25mM glucose medium. We also investigated the difference on glucose uptake between insulin-sensitive individuals and patients affected by Type 2 Diabetes.
Conclusion: We developed a microfluidic platform for culturing small-scale human adipose tissue and allowing to accurately control the temporal evolution of the culture conditions in terms of concentration of metabolites, oxygen, and insulin concentration. This system with in line biosensors open important perspectives towards the realization of high-throughput dynamic screening of anti-diabetic drugs on human adipose tissue.
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Effects of different commercial insulins on adipogenesis and adipocyte metabolism
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Background and aims: Insulin has several roles in metabolism, so the use of exogens insulins could be modifying metabolic process related with adipo-

cyte metabolism that might explain the secondary problems derivated of insulin therapy. We investigate the effects of six different insulins on adipocyte cell differentiation, lipolytic activity, and expression levels of PPARg, SCD-1, HSL, InsR and SREBP-1c .
Materials and methods: 3T3-L1 cells were induced to differentiate with 6 commercial insulins: Glargine, Lispro, Aspart, Detemir, NPH and regular recombinated human insulin (used as control). Cell differentiation, lipolysis and gene expression were measured at day 7 (D7) and at day 10 (D10) after differentiation induction.
Results: The highest values of cell differentiation and lipolisys were found at D10 for all the insulins tested ($p<0.001$). Preadipocyte differentiation was different according to the insulin used at both moments ($p<0.0001$), Detemir insulin being the least adipogenic. PPARg mRNA level variated according to the insulin and it was a good genetic marker of adipogenesis at D7, but at D10 PPARγ gene expression didn't reflect the differences on the cell differentiation between each insulin. Cells treated with Glargine insulin showed lower antilipolytic effects ($p<0.0001$) with the highest level of HSL expression at both days ($p< 0.0001$ at both days). Gene expression of InsR, SREBP-1c and SCD-1 were different regarding to the insulin investigated ($p<0.0001$, $p<0.0001$and $p<0.001$ respectively at D7; and $p<0.001$, $p<0.003$ and $p<0.005$ respectively at D10)
Conclusion: Detemir insulin is less adipogenic than the other insulins tested, while Glargine insulin is the less anti-lipolitic. The modifications made on commercials insulins also affect the adipocyte differentiation, the lipolysis activity, and the expression of different genes which can modify metabolic pathways independent of glucose metabolism.
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Orexin A stimulates lipogenesis and adiponectin expression via PPARgamma-dependent mechanism in isolated primary rat and mature 3T3-L1 adipocytes
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Background and aims: Orexin A (OXA) plays a role in the regulation of food intake and body metabolism. Dysfunction of the orexin system is associated with obesity and glucose intolerance. Recently, orexin receptors were identified in human adipocytes. Activation of orexin receptors in adipocytes increased PPARgamma2 expression and reduced lipolysis. Here, we characterize the effects of OXA on glucose uptake, lipid accumulation, adiponectin gene expression, and secretion in adipocytes. We also describe the underlying mechanism.
Materials and methods: We used isolated primary rat adipocytes and differentiated 3T3-L1 adipocytes. We studied the effects of OXA on lipogenesis, lipolysis, glucose uptake and ATP levels using biochemical assays, Western blots and immunofluorescence. The effects of OXA on adiponectin secretion and expression were measured by RIA and Western blots. Mechanisms of action were studied using siRNA technique and pharmacological inhibitors of crucial signaling pathways.
Results: OXA stimulated active glucose uptake by translocating the glucose transporter type 4 from plasma into the plasma membrane via the PI3kinase-/AKT-pathway. OXA increased cellular ATP content, as well as lipid accumulation. OXA enhanced the lipogenesis and reduced lipolysis. OXA increased adiponectin secretion and expression. OXA increased the expression of PPARgamma. The effects of OXA on lipogenesis and adiponectin secretion were blocked by pharmacological inhibitors of PPARgamma activity and by specific PPARgamma siRNA.
Conclusion: In summary, our study demonstrates that OXA PI3kinase-/AKT-dependently stimulates glucose uptake in adipocytes and that the evolving energy is stored as lipids. OXA increases the expression and secretion of adiponectin, as well as lipogenesis through PPARgamma-dependent mechanism. The effects of OXA on the function of adipocytes may be of clinical relevance in the pathophysiology of obesity and in peripheral insulin resistance, the hallmark of type 2 diabetes mellitus.
Supported by: DFG

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Carrier-mediated trans-membrane delivery of PIP_3 overcomes cellular insulin resistance induced by proximal, but not distal signaling defect
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Background and aims: Using bio-mimetic liposomes we have identified an efficient specific polyethyleneimine (PEI)-based carrier to efficiently deliver phosphoatidylinositol-3,4,5-trisphophate (PIP_3) across the plasma membranes of cultured muscle and fat cells in a manner that can activate insulin signaling (Diabetologia, 51: S276: 687). In the present study, we aimed at obtaining proof-of-concept for the possibility to utilize PIP_3 to overcome some forms of insulin resistance.
Materials and methods: We utilized chronic exposure of L6 muscle cells to high glucose-high insulin to induce an insulin resistance state characterized by early signalling defect, and 3T3-L1 adipocytes treated with nelfinavir as a cellular model for insulin resistance emanating from signalling defect(s) downstream of endogenous PIP_3 generation (Endocrinology 150: 2618, 2009).
Results: 3T3-L1 adipocytes treated for 18h with the HIV protease inhibitor nelfinavir exhibited marked attenuation of insulin-stimulated translocation and plasma membrane fusion of a GFP-GLUT4-myc reporter. PEI-PIP_3 complexes induced in control cells a nearly full response of GLUT4 translocation compared to that observed with insulin. Yet, after pre-incubation with nelfinavir, the response to PEI-PIP_3 was greatly blunted, suggesting that PEI-PIP_3 can not bypass the signaling defect induced by nelfinavir. In contrast, L6 cells exposed for 18h to high (25 mM) glucose high (100nM) insulin exhibited a 2-fold increase in basal Ser473 phosphorylation of Akt, along with 40% diminished insulin-stimulated Akt phosphorylation. In this system, control cells treated with PEI-PIP_3 exhibited 70% of the 10-fold increase in pS-Akt induced by insulin, and after high-glucose - high insulin treatment the response to PEI-PIP_3 was fully intact. Moreover, pS-Akt was higher after high-glucose - high insulin in response to PEI-PIP_3 than in response to insulin.
Conclusion: We show that efficient carrier-mediated delivery of PIP_3 can elicit biologically relevant insulin-like responses in adipocytes and muscle cells. Insulin resistance resulting from signaling defects downstream of endogenous PIP_3 generation can not be bypassed by introducing exogenous PIP_3. However, PEI-PIP_3 retains its ability to elicit insulin signaling events in muscle cells rendered resistant to the effects of the hormone when the signaling defect is upstream of PIP_3 generation, constituting a proof-of-concept for "second messenger therapeutics" for some forms of insulin resistance.
Supported by: Israel Science Foundation

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Immunosuppressive agents alter insulin signalling and glucose and lipid metabolism in human subcutaneous adipocytes
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Background and aims: The immunosuppressive agents (IAs), Cyclosporine (CsA) and Tacrolimus (FK) can cause new-onset diabetes in transplant recipients (NODAT). This is one of the most serious long-term metabolic complications of solid organ transplantation and it is associated with increased cardiovascular morbidity and mortality. Rapamycin (Rap), another potent IA and potential anti-tumoral agent, inhibits the mammalian target of rapamycin (mTOR). Although mTOR can mediate nutrient-induced insulin resistance by down-regulating insulin receptor substrate proteins, with subsequently reduced AKT phosphorylation, the effects of Rap on glucose metabolism are unclear. The aim of this study was to elucidate direct effects of IAs on insulin signaling, glucose and lipid metabolism in human subcutaneous adipocytes.
Materials and methods: Abdominal subcutaneous adipose tissue was obtained from healthy volunteers (n=22, BMI: 21-36kg/m^2). After adipocyte isolation, cells were incubated in medium containing 4% albumin with or without IAs: CsA (0.001-10 μM); FK (0.001-10 μM) and Rap (0.001-10 μM),

all ranges including therapeutic levels. Following adipocyte incubation with or without IAs, ^{14}C-Glucose uptake was measured (75 minutes incubation, n=7) and western-blot analyses of insulin signaling protein (IRS1/2, PI3K, GLUT4, AKT and insulin-stimulated pSer473AKT and pThr308AKT) were performed (n=13; 15 minutes, 3 and 24 hours incubation). Basal and isoprenaline-stimulated lipolysis rates were measured after adipocyte incubation with IAs for 2 hours (n=15).
Results: Insulin-stimulated glucose uptake was reduced in a dose-dependent manner after incubation with IAs: CsA by 23-28% ($p<0.001$), FK by 23-43% ($p<0.001$) and Rap by 12-42% ($p<0.001$). Treatment with Rap (≥3 h) caused a reduction in IRS2 protein level by 20% ($p<0.01$). FK and Rap caused a decrease in insulin-stimulated phosphorylation of AKT Ser473 ($p<0.05$), but did not change IRS1, PI3K, AKT and GLUT4 protein levels. Exposure of cells to CsA, FK and Rap increased isoprenaline-stimulated lipolysis (1.7, $p<0.01$; 1.5, $p<0.05$ and 1.2 fold, $p<0.05$, respectively) but only Rap induced basal lipolysis (1.4 fold, $p<0.05$).
Conclusion: These results demonstrate that the investigated IAs, CsA, FK as well as the mTOR inhibitor Rap, impair insulin action on glucose uptake in subcutaneous human adipocytes, and this may be mediated via increased lipolysis and altered insulin signaling. These alterations can contribute to the development of NODAT and to dyslipidemia found in post-transplant patients. Moreover, it is suggested that mTOR is an important modulator of insulin-stimulated glucose uptake. Inhibition by Rap may contribute to the development of NODAT in organ transplanted patients, rather than having an insulin-sensitizing effect.
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Metabolic endotoxaemia as a mediator of mitochondrial dysfunction in human adipose tissue, alleviated by salicylate
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Background and aims: The pathogenesis of obesity and type 2 diabetes (T2DM) mediates mitochondrial dysfunction which, in part, may arise from a chronic low level inflammatory response due to gut derived endotoxaemia. Our previous studies have shown that changes in diabetic status increase circulating endotoxin levels. Furthermore our current studies have shown that serum endotoxin levels correlate positively with BMI ($r=0.281$, $p=0.016$) as well as serum insulin levels ($r=0.34$, $p=0.009$) and HOMA index ($r=0.331$, $p=0.021$) suggesting a positive association between circulating endotoxin levels and insulin resistance. As previous studies in muscle from patients with severe IR have highlighted decreased mitochondrial function, we undertook studies to investigate whether endotoxin may, in part, influence mitochondrial dysfunction in human adipose tissue (AT).
Materials and methods: Abdominal subcutaneous (Abd Sc), omental (Om) (BMI: 20.1-33.9kg/m^2, age: 29-48yr; n=35) and Abd Sc T2DM (BMI: 52.4-67.59kg/m^2; age: 29-44yr; n=7) AT was taken from subjects undergoing elective surgery with ethical approval. Gene expression was assessed by qRT-PCR.
Results: In Abd Sc AT, increasing adiposity significantly reduced FAS ($p<0.05$), COX4 and UCP2 mRNA ($p<0.001$), whilst PGC1α mRNA was increased ($p<0.01$). No significant difference in expression was observed in Abd Sc AT from T2DM subjects vs. Abd Sc AT from obese ND subjects. In Om AT, FAS, PGC1α, COX4 and UCP2 mRNA were reduced ($p<0.001$). No significant difference in expression was observed in Abd Sc AT from T2DM subjects vs. Abd Sc AT from obese ND subjects. Therefore we investigated the direct effect of endotoxin (lipopolysaccharide (LPS)) treatment on mitochondrial encoded genes and the therapeutic potential of Salicylate (Sal) in human differentiated adipocytes. Differentiated pre-adipocytes were treated for 24 hr with LPS (100ng/ml) with or without Sal (20mM) and assessed by qRT-PCR. Treatment with LPS+Sal led to a significant up-regulation of NRF1, FAS, UCP2 and UCP3 ($p<0.05$) compared with adipocytes treated with LPS alone.
Conclusion: In summary, these studies highlight significant changes in mitochondrial properties in response to conditions of obesity and T2DM. Such dysregulation in mitochondrial gene expression may, in part, arise due to the effects of inflammation imposed by LPS which appears negated by Sal treatment. Taken together, these data indicate therapeutics to reduce LPS may alleviate mitochondrial dysfunction and its pathogenic consequences.
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The effect of ligand activated LXR-alpha on the PPAR-γ-target genes in mature adipocytes
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Backgrouds and aims: A selective activation of peroxisome proliferator-activated receptor gamma (PPAR-γ) in adipocytes plays an important role in insulin sensitivity and inflammation. Like PPAR-γ, Liver X receptor alpha (LXR-α), which is another ligand-activated transcription factor in adipocytes, also forms a heterodimer with retinoid X receptor alpha (RXR-α). Whether the activation of LXR-α interferes with the PPAR-γ signaling in adipocytes is unknown. To explore potential interactions between LXR-α and PPAR-γ in adipocytes and investigate the effect of LXR-α activation on the expressions of PPAR-γ target genes.
Methods: Differentiation of pre-adipocytes 3T3-L1 into mature adipocytes were induced by addition of differentiation cocktail for 12 days, and cells were harvested every 2 days for LXR-α mRNA analysis. T0901317 was used to activate LXR-α or Pioglitazone to activate PPAR-γ respectively. Differentiated adipocytes (at day 8) were treated with (1) different concentrations of T0901317 (0, 0.3, 1, 3, 6 and 10uM) for 24 h; (2) different concentrations of T0901317 with 3uM Pioglitazone for 24 h; (3) LXR-α silence (or non-silencing control) for 48 h followed by different concentrations of T0901317 for 24 h; (4) LXR-α silence (or non-silencing control) for 48 h followed by different concentrations of T0901317 with 3uM Pioglitazone for 24 h. Expressions of the PPAR-γ target genes in harvested adipocytes, including adiponectin, resistin and TNF-α, were determined by Real-time_PCR, and Western blot.
Results: (1) LXR-α mRNA level increased dramatically 4 days after the initiation of the differentiation program, reached the peak at day 8 (16.3 times of that in pre-adipocytes 3T3-L1) and gradually declined at day 10 and day 12. (2) Adiponectin, a representative gene product positively regulated by PPAR-γ, was suppressed dose dependently by T0901317 in both the mRNA and the protein levels. mRNA levels of TNF-α and resistin produced by genes negatively regulated by PPAR-γ were increased dose dependently by T0901317. Addition of pioglitazone at a concentration of 3uM partly reversed the expressions of adiponectin, resistin and TNF-α at each concentration of T0901317. However, the dose dependent suppressions of adiponectin and up-regulations of TNF-α and resistin transcriptions by T0901317 were still observed in the presence of pioglitazone. (3) Treated with shRNA specific for LXR-α for 48 h, the differentiated adipocytes were down-regulated by 60-70% in LXR-α protein level. mRNA level of adiponectin was moderately increased, while TNF-α and resistin mRNA levels were decreased by LXR-α silencing at each concentration of T0901317. More significant increase in adiponectin, as well as decrease in TNF-α and resistin mRNA levels were found in presence of 3uM pioglitazone,as compared with those treated with non-silencing control shRNA and different concentrations of T0901317; However, the absolute increase in adiponectin mRNA and reduction in resistin and TNF-α mRNA levels by LXR-α silencing were decreased consistently with the increasing concentration of T0901317, regardless of the presence of 3uM Pioglitazone.
Conclusion: These observations suggest that LXR-α activation interferes with the expressions of PPAR-γ-target genes, indicating a possible role of LXR-α in insulin action by counteracting PPAR-γ signaling in adipocytes.

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PPARγ-sparing thiazolidinediones: stimulation of mitochondrial biogenesis by a PGC1α-independent pathway
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Background and aims: It is well established that mitochondrial content and capacity are decreased in some tissues in insulin-resistant states and type 2 diabetes. Therapeutic agents such as the thiazolidinediones (TZDs) have shown promise in restoring mitochondrial capacity in both experimental models and clinical studies. However, the TZDs have well established side effects which can be traced back to activation of the PPARγ transcription factor. Therefore, we have sought to identify and develop experimental drugs of the TZD class that spare this receptor (PPARγ-sparing thiazolidinediones, PsTZDs) but retain the desirable pharmacology, including stimulation of mitochondrial biogenesis.
Materials and methods: We have used the PsTZD clinical candidate, MSDC-0160, to examine mitochondrial biogenesis in cells derived from brown ad-

ipose tissue (BAT). In this model system, BAT precursor cells are isolated from CD1 mice and maintained in tissue culture.
Results: Treatment of cells with MSDC-0160 elicited the differentiated BAT cellular phenotype after 96 hours of drug treatment. During this timeframe, a robust increase in mitochondrial content was observed as measured by fluorescent dye staining, light microscopy and confirmed by transmission electron microscopy. Expression of the mitochondrial uncoupling protein, UCP1 (mRNA, protein) was first detected following 96 hours of drug treatment and maximum expression was observed after 168 hours. Citrate synthase was monitored as an index of mitochondrial mass and a detailed time course of expression after drug treatment revealed progressive, dose-dependent increases (4 to 5-fold). We assumed that the nuclear transcription factor, PGC1α (peroxisomal proliferator activator receptor γ coactivator 1α), was driving the increased mitochondrial content through expression of nuclear and mitochondrial genes involved in mitochondrial biogenesis. However, analysis of PGC1α mRNA expression as a function of time of drug treatment revealed that there was no detectable increase. We then examined the ability of the PsTZDs to elicit differentiation and mitochondrial biogenesis in BAT precursor cells isolated from mice in which the PGC1α gene had been ablated. We found that cellular differentiation, UCP1 protein expression and mitochondrial biogenesis as measured by citrate synthase activity were robust and similar to that seen in the control animals.
Conclusion: Therefore, we conclude that the PsTZDs are fully capable of stimulating mitochondrial biogenesis through a pathway that does not include PGC1α. This pathway may synergize with the epinephrine/PGC1α pathway. The definition of this PsTZD-stimulated, PGC1α -independent pathway may have important implications for understanding the mechanism of action of insulin sensitizing agents.

PS 60 Glucose and lipid metabolism in animal models

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Insights into the mechanism of FATP1 activating effect on pyruvate dehydrogenase

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Background and aims: FATP1 is a membrane-bound fatty acid (FA)-binding protein that has acyl-CoA synthetase activity. FATP1 gene expression is highest in skeletal muscle, heart and fat. FATP1 is a metabolic regulator. FATP1-null mice are protected against diet-induced obesity and insulin resistance. In cultured skeletal muscle cells, FATP1 overexpression enhances FA uptake and storage as triacylglyceride, whereas FA oxidation is either not stimulated or only moderately enhanced. FATP1 strongly stimulates glucose oxidation and raises the activity of the pyruvate dehydrogenase (PDH) complex and pyruvate decarboxylase PDH-E1 catalytic subunit. FATP1 is immunolocalized in internal membranes and mitochondria, although the suborganelle compartment is unknown. We examined the mechanism of effect of FATP1 on PDH activity and submitochondrial localization of FATP1 in cultured skeletal muscle cells.
Materials and methods: FATP1 effects on PDH activity were analyzed after adenoviral-mediated transfer of the mouse FATP1 cDNA into cultured muscle cells. PDH complex and PDH-E1 subunit activities were determined by measuring the $^{14}CO_2$ production from [1-^{14}C]-pyruvate. Western blotting was used to assess PDH complex protein content and phosphorylated PDH-E1α at site 1 and site 2 using specific antibodies (kindly provided by Dr. H. Pilegaard). Subcellular and suborganelle immunolocalization was assessed in cells expressing FATP1 fused at the C terminus to EGFP using anti-GFP antibodies and electron microscopy.
Results: Overexpression of FATP1 in cultured myotubes raised the levels of the active form of the PDH complex and the PDH-E1 catalytic subunit, whereas no differences in the phosphorylation of PDH-E1α at site 1 or site 2 were observed. The levels of the active form of the PDH complex and the PDH-E1 catalytic subunit were not altered by incubation of myotubes with palmitate, oleate or their mixture. Palmitate partially counteracted the activation of the PDH complex and PDH-E1 catalytic subunit by FATP1, whereas oleate or the mixture of palmitate with oleate did not. Immuno-electron microscopy showed that FATP1-GFP was localized inside the mitochondria, within the inner membrane-matrix compartment, whereas GFP was localized intracytoplasmatically and in the nucleus.
Conclusion: In cultured skeletal muscle cells FATP1 activates the PDH complex and PDH-E1 catalytic subunit, without affecting the phosphorylation of PDH-E1α at site 1 or site 2. This activation is counteracted by palmitate. FATP1-GFP is localized in skeletal muscle cells inside the mitochondria, where the PDH complex lies. These data provide insight into how FATP1 activates the PDH complex.
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Adipose tissue-specific cholesteryl ester transfer protein expression in mice: impact on glucose metabolism

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Background and aims: Cholesteryl ester transfer protein mediates HDL cholesteryl ester delivery to the liver cells. Adipose tissue is a highly conserved site of cholesteryl ester transfer protein (CETP) expression across species, adipose tissue CETP makes a major contribution to CETP in the circulation. To investigate the impact of adipose CETP expression on adipocyte function and metabolism of glucose and lipid, we set up adipose tissue-specific CETP transgenic (CETPTg) mice.
Materials and methods: We created adipose tissue-specific CETP transgenic (CETPTg) mice successfully, using the aP2 promoter. HE-stained sections of adipose tissue from CETPTg mice and WT mice, in order to know the

change of adipocyte size, the frequency distribution of adipocyte cell surface area of both groups were evaluated. Then we evaluated the body weight and food consumption after four month chow diet or high fat diet. The glucose tolerance test and insulin tolerance test were all applied in CETPTg mice and WT mice after chow or high fat diet.
Results: CETP mRNA was predominantly expressed in adipose tissue in CETPTg mice. Plasma lipoprotein analysis showed marked reductions in HDL cholesterol ; and adipocytes were significantly smaller than those in control mice and stored less lipid than those of wild type mice. No differences were found between WT and CETPTg mice in body weight and food consumption after four month chow diet or high fat diet. Differences between the CETPTg and the WT littermates under chow diet were detected when a glucose tolerance test was administered. The blood glucose levels in the CETP Tg mice were higher than in WT mice after injection of a glucose solution. The differences were significant after 30 min and 60min of the test ($P< 0.05$), indicating that CETPTg mice displayed impaired glucose tolerance. In an insulin tolerance test, the CETPTg mice became significantly more hypoglycemic at 15, 30min after insulin injection than the WT mice ($P < 0.001$, $P < 0.05$ respectively), showed an increased sensitivity to insulin. The GTT and ITT were also investigated in the CETPTg and WT mice after high fat diet. The results indicated that the tendency was still there but without significance, high fat diet masked the effect of CETP overexpression.
Conclusion: GTT reflects both insulin sensitivity and insulin secretion, while ITT only reflects insulin sensitivity. Smaller adipocyte shows a higher insulin sensitivity. Adipose tissue-specific CETP overexpression reduced their adipocyte size with an increased insulin sensitivity, however the glucose tolerance was impaired. We predict that maybe there is problem with the beta cell function, we need to do further investigation in the next study.
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Loss of pigment epithelium-derived factor deteriorates lipid and glucose metabolism
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Background and aims: Pigment epithelium-derived factor (PEDF) is an endogenous protein with neuroprotective and anti-angiogenic effects. Experimental studies report that PEDF is a putative ligand for adipose triglyceride lipase. Clinical reports indicate that elevated serum PEDF may be a compensatory protein in the metabolic syndrome. However, the physiological significance of PEDF as a regulator of lipid metabolism is unknown. We hypothesized that deletion of PEDF promotes lipid accumulation and leads to insulin resistance
Materials and methods: PEDF deficient (PEDF ko) and age-matched control (WT) mice (n=8/group) were studied in metabolic cages on a regular chow diet, and an IPGTT (1 mg/g) was performed after an overnight fast.
Results: Compared to WT mice, energy expenditure was reduced 25% ($p<0.001$) in PEDF ko mice with a 50% decrease in food consumption ($P<0.01$) and total activity ($P<0.001$). Body weight was increased by 10% ($P=0.01$), and fat mass, as measured by 1H magnetic resonance spectroscopy, was more than two-fold higher ($P<0.001$) in PEDF deficient mice. PEDF ko mice had higher fasting glucose concentrations (156±8 vs 103±8 mg/dL, $P<0.001$), and marked hyperinsulinemia (58±2 vs 14±3 μU/ml, $P=0.02$), suggesting severe insulin resistance. During the IPGTT, the glycemic excursion was higher in the PEDF ko mice with an 80% increase in the AUC glucose, but without additional glucose stimulated insulin secretion.
Conclusion: Loss of PEDF promotes obesity and insulin resistance in mice.
Supported by: DFG

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Effect of RNAi-mediated gluconeogenic gene depletion on the fasting blood glucose level in alloxan-diabetic mice
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Background and aims: Glucose 6-phosphatase(G6Pase) catalyses the final reaction in hepatic glucose production by gluconeogenesis, and has been proposed as a potential target for antihyperglycaemic drugs for type 2 diabetes. PPAR gamma-coactivator 1(PGC-1) stimulatse this enzyme. To evaluate the potential of G6Pase and PGC-1 as a therapeutic target of type 2 diabetes, we investigated the response to dietary caloric restriction on the PGC-1 expression in liver of Otsuka Long-Evans Tokushima Fatty(OLETF) rats, and perfomed depletion of G6Pase and PGC-1 gene by siRNA-expressing plasmid DNA(siRNA-pDNA) injection in alloxan-diabetic mice.
Materials and methods: In OLETF rats and Long-Evans Tokushima Otsuka(LETO) rats, liver PGC-1 mRNA and blood glucose levels were investigated at 1, 2, and 3 weeks after the beginning of 30% caloric restriction(CR). siRNAs of G6Pase and PGC-1 were constructed to siRNA-pDNA. Each siRNA-pDNA was injected to alloxan-diabetic mice by tail vein and monitored blood glucose level. The levels of G6Pase and PGC-1 mRNA in liver of mice were measured by real time PCR.
Results: The liver PGC-1 mRNA expression were increased to 19% in LETO rats but significant change was not observed in OLETF rats by 30% CR. Postprandial blood glucose level was not changed between control and siRNA-pDNA(G6Pase, PGC-1) treated alloxan-diabetic mice. After 12 hour fasting, the blood glucose levels of control were 235±27 mg/dL. Each glucose levels of G6Pase and PGC-1 siRNA-pDNA injected alloxan-diabetic mice were 115±16 and 127±10 mg/dL at the same time. There were significant differences between control and experimental groups after 12 hour fasting. G6Pase and PGC-1 mRNA levels in liver of mice were decreased by injection of siRNA-pDNA.
Conclusion: Gluconeogenic gene such as G6Pase and PGC-1 depletion by siRNA-pDNA treatment leads to lowering fasting blood glucose levels in diabetic mice. This represents the potential of G6Pase and PGC-1 as a therapeutic target of type 2 diabetes.

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Effects of high-fat diet on lipid metabolism in ApoE-/- mice
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Background and aims: High-fat diet (HFD) is associated with insulin resistance, hyperinsulinemia, elevated plasma free fatty acid (FFA), and increased risk for atherosclerotic vascular disease (ASVD). However, the mechanisms underlying the tissue-specific effects of HFD on the expression of genes involved in glucose and lipid metabolism have not been fully clarified. In our study, we have studied the effects of HFD on key enzymes and transcription factors involved in glucose and lipid metabolism in $ApoE^{-/-}$ mice.
Materials and methods: Twenty male $ApoE^{-/-}$ mice aging 8 weeks old were housed in individual cages and subjected to an environmentally controlled room with a 12-h light/dark cycle. Mice were randomly assigned to one of two groups. One for a normal chow diet (NC, n=10). The other for a high-fat diet (HF, n=10). Hyperinsulinemic-euglycemic clamp study was carried out. Plasma FFA, insulin concentrations (PIns), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), TG and TC concentrations were measured. The mRNA levels of insulin-induced gene (INSIG), sterol regulatory element binding protein cleavage activating protein (SCAP), sterol regulatory element binding protein-2 (SREBP-2), 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) and low density lipoprotein receptor (LDLr) were determined by RT-QPCR; The concentrations of protein visfatin and FGF-21 were assay. The liver and adipose in mice ATGL, $INSIG_2$, FGF-21 and visfatin protein were measured by western blot.
Results: Whole body insulin sensitivity were evaluated by hyperinsulinemic-euglycemic clamp technique combined with [3-^{3}H] glucose as a tracer. The GIR in the HF group was markedly lower than in the controls ($P< 0.01$).Compared to controls, mice fed a high fat diet (HFD) had significantly increased body weight, fasting blood glucose (FBG) and plasma concentrations of insulin, FFA, TG, TC, HDL-C, and LDL-C ($P<0.01$,). The mRNA expression

of INSIG2 and SCAP was significantly up-regulated ($P<0.01$,) and INSIG2 protein level in liver was also increased in HF group ($P<0.05$). The mRNA expression of SREBP-2, HMGCR and LDLr was down-regulated in liver of HF group ($P<0.05$ and $P<0.01$). In adipose tissue, mRNA expression of INSIG1, INSIG2 and SCAP, and protein levels of INSIG2 were no differences between the two groups. The mRNA expression of SREBP-1, ATGL and PPARγ in liver and adipose tissues were significantly down- regulated in HF group than in NC group ($P<0.05$ and $P<0.01$). But FAS mRNA and HSL levels were no differences in two groups.
Conclusion: Hyperglycemia, dyslipidosis and hyperinsulinemia in this animal model might result from, at least in part, the changes of these key enzymes and transcription factors. Our findings might also account for the characteristics of glucose and lipid metabolism in diet-induced insulin resistance.
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The lack of beta 2 adrenoceptors in mice results in glucose intolerance and impaired insulin secretion
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Background: The reciprocal regulation of Sympathetic Nervous System and Insulin relays on molecular mechanisms that are not fully understood. Beta 2 adrenergic receptors (beta 2 ARs), in particular, are known to be involved in insulin production and in peripheral glucose uptake, but their role in development of diabetes is not clear.
Methods and results: We characterized the metabolic phenotype of mice (age 6 months) with deletion of the beta 2 AR gene (KO, n=21), performing tolerance tests to glucose (GTT, 1mg/Kg), insulin (ITT, 0.75 IU/Kg) and sodium pyruvate (PTT 2g/Kg) and hyperinsulinemic euglicemic clamps. As control we used C57BL/6 mice (WT, n=18). KO mice exhibited decreased glucose tolerance compared to WT mice (AUC GTT KO: 40980±6890 vs AUC GTT WT 19290±2120, p<0.01, ANOVA). However, ITTs showed that KO mice did not feature peripheral insulin resistance compared to WT mice (AUC ITT KO: 8280±1250 vs AUC ITT WT 13950±1815, p<0.01, ANOVA). Clamp studies in KO mice revealed a lower Glucose Infusion Rate (KO vs WT: 14.6±8 vs 30±9 mg/Kg/min), an increased endogenous glucose production: (79.8±4 vs 36.1±3 mg/Kg/min) and an increased rate of glucose disappearance (96.6±5 vs 63.2±3.4 mg/Kg/min; p<0.05), suggesting that insulin resistance develops at the hepatic level in KO mice compared to WT mice. KO mice showed impaired insulin response to hyperglycemia compared to WT mice. Histological analysis of pancreatic tissues displayed a normal architecture of the islets in both KO and WT mice. Also total insulin content (insulin KO: 4.93±0.87 ng/g of pancreas vs WT: 4.19±1.82 ng/g of pancreas) and glucagone content (glucagone KO: 577.8±106.5 pg/g of pancreas vs WT: 636.17±112.8 pg/g of pancreas) were comparable in both KO and WT mice. However, whilst in islets isolated from WT mice glucose induced insulin secretion by 3-fold, islets from the KO mice exhibited a blunted response to glucose. Glucagon secretion was not different in KO and WT mice. Increasing beta 2 AR expression in KO mice isolated islets by the infection of an adenoviral construct bearing the beta 2 AR cDNA, restored the ability of KO islets to secrete insulin in response to glucose.
Conclusion: Our results suggest that beta 2 AR plays a critical role in the regulation of glucose metabolism and insulin secretion both in vivo and ex vivo. These findings open new fields of investigation for the development of strategies to produce drugs with potential therapeutic applications in diabetes.

PS 61 Animal models insulin resistance

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Folate deficiency increases adipose tissue and muscle insulin resistance in spontaneously hypertensive rats
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Background and aims: Controversial clinical data and a few studies in animal have been reported on the association between insulin resistance and plasma folate levels. The aim of this work was to compare the influence of the folate-deficient diet and high-fructose diet on insulin resistance parameters in spontaneously hypertensive rats (SHR), which are commonly used as a model of a metabolic syndrome.
Materials and methods: One group of adult male SHR rats (n=9) were fed a folate-deficient diet (Harlan Teklad, Netherlands) *ad libitum* for 4 weeks, the second group (n=9) were fed a high-fructose diet (70 cal.%). Triglycerides, glucose and free fatty acids concentrations were determined with commercially available kits (Pliva Lachema, Czech Republic; Roche Diagnostic GmbH, Germany, resp.). Serum folate levels were estimated by folate test AxSYM (Abbott Laboratories). Tissue insulin sensitivity was measured *in vitro* without or with insulin (250 μU/ml) according to basal and insulin-stimulated ^{14}C-U-glucose incorporation into muscle glycogen or adipose tissue lipids.
Results: After 4 weeks, the rats fed the folate-deficient diet has lower serum folate concentration in comparison with rats fed a high-fructose diet (11.07±0.47 nmol/l *vs* 75.96±3.46 nmol/l; p<0.00001). Animals fed with folate-deficient diet showed increased body weight (381±4.9 g *vs* 351±6.1 g; p<0.001), whereas epididymal fat pads weight when related to body weight were not different between groups. Nonfasting serum glucose levels were increased in the folate deficient rats (5.8±0.1 *vs* 4.99±0.2 mmol/l; p<0.0004). Surprisingly folate-deficient diet markedly decreased serum triglycerides concentrations (0.77±0.03 *vs* 2.14±0.17 mmol/l; p<0.000001) whereas free fatty acids were not different. There were found no significant differences in liver and muscles triglycerides concentrations between folate-deficient or fructose fed rats, however both groups showed hepatic steatosis as a results of increased triglycerides accumulation (17.69±1.30 *vs* 15.46±0.98 μmol/l, N.S.). The rats fed on the folate-deficient diet exhibited significantly decreased glucose incorporation into adipose tissue lipids and into muscle glycogen in comparison with fructose fed animals (Tab). The ability of insulin to stimulate glucose incorporation was lower in folate-deficient rats than in fructose fed rats.
Conclusion: Our results show that folate-deficiency impaired glucose sensitivity of peripheral tissues and stimulated hepatic steatosis similarly as fructose feeding and support hypothesis that folate deficiency may contribute to the pathogenesis of metabolic syndrome.

		Folate-deficiency diet	High-fructose diet	p<
Lipogenesis	-insulin	338 ± 42	467 ± 46	0.05
(nmol glucose/g/2h)	+insulin	738 ± 89	1427 ± 221	0.01
Glycogenesis	-insulin	39 ± 5,7	299 ± 60	0.0005
(nmol glucose/g/2h)	+insulin	68 ± 8,7	644 ± 62	0.0000001

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Enhanced neuronal NO Synthase breakdown through the ubiquitin-proteasome pathway in skeletal muscle of insulin resistant Zucker *fa*/*fa* rats
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Background and aims: In type 2 diabetes, due to a decrease in insulin sensitivity, glucose uptake is impaired in skeletal muscle. The latter is well known to express a splice variant of the neuronal isoform of NO Synthase (nNOS), nNOSμ. Because nNOSμ exerts a physiological role in the control of skeletal

muscle contractility and glucose uptake, we were prompted to investigate whether alterations of muscle nNOSμ could occur in prediabetic states and in the early phases of type 2 diabetes.
Materials and methods: We performed our study using Zucker *fa*/*fa* rats, characterized by a strong insulin resistance and hyperinsulinemia, two important features of prediabetic states. Zucker *fa*/+ lean rats were used as controls. nNOSμ catalytic activity was evaluated by measuring citrulline production from labelled arginine. nNOSμ expression was measured by quantitative PCR and western blotting. MG132 was used as an inhibitor of proteasomal function. Subcellular localization of nNOSμ was determined in immunofluorescence studies with specific antibodies.
Results: In skeletal muscle extracts from Zucker *fa*/*fa* rats, we found a 41% decrease in nNOSμ catalytic activity when compared to control *fa*/+ extracts ($p<0.01$). This decrease correlates with a significant 42% reduction in the enzyme proteic level ($p<0.05$) with no change in nNOSμ mRNA, which argues for the occurrence of an increased proteasomal breakdown. Use of the proteasomal function inhibitor MG132 in isolated skeletal muscle enabled us to bring evidence for an increased level of nNOSμ ubiquitination in *fa*/*fa* rats. In addition, inhibition of the ubiquitin-proteasome pathway resulted into a significant recovery of both nNOSμ proteic expression and catalytic activity, to levels similar to those recorded in *fa*/+ controls. In immunofluorescence studies, we could confirm the decrease in the expression nNOSμ protein in skeletal muscle of *fa/fa* rats; interestingly this decrease was found associated to a disturbance of the enzyme sub-membrane distribution.
Conclusion: In Zucker *fa/fa* obese insulin resistant rats, skeletal muscle displays a decreased nNOSμ catalytic activity resulting from an increased breakdown of the enzyme through the ubiquitin-proteasome pathway. These abnormalities, associated to a disturbed nNOSμ sub-membrane distribution, could be involved in the impaired skeletal muscle glucose uptake in the early phases of type 2 diabetes.

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Analysis of insulin sensitivity in $p66^{shcA}$ KO mice by hyperinsulinaemic euglycaemic clamp
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Introduction and aim: Emerging clinical and experimental evidence point to a major role of tissue oxidative stress in linking ageing and excess body weight with insulin resistance; molecular mechanism underlying this connection are however still elusive. The signaling adaptor protein $p66^{shcA}$ promotes mitochondrial generation of oxidant species in several models of age-related disease, and p66KO mice live 30% longer than their WT littermates; thus, this protein may represent a critical determinant of the intracellular redox-state imbalance associated with diabetes and metabolic syndrome. We have previously shown that genetically obese, hyperinsulinemic ($Lep^{Ob/Ob}$) mice lacking p66shcA are partially protected from glucose intolerance observed in their p66shc proficient littermates (Chiatamone et al., submitted), demonstrating that p66shc promotes insulin resistance in this setting. However, no major effects of p66shc on glucose homeostasis were detected in normal, lean mice by standard Intraperitoneal glucose tolerance test (ipGTT, 1g glucose/Kg BW) and insulin tolerance test (ITT, 0.75 U/Kg BW).
Methods and Results: Prompted by this discrepancy we have set up and performed hyperinsulinemic euglycemic clamp (18 $mUI \cdot kg^{-1} \cdot min^{-1}$) on lean mice of the two genotypes. This more reliable assay clearly demonstrated an enhanced insulin sensitivity in $p66^{shcA}$-/- mice in comparison to WT animals (M, as mg of glucose metabolized per kg of BW per minute, and SEM: respectively 161 $mg \cdot kg^{-1} \cdot min^{-1}$,± 35; 66 $mg \cdot kg^{-1} \cdot min^{-1}$,± 4.4; $p<0.02$). In keeping with this finding, biochemical analysis of the insulin signalling pathway in the adipose tissue of $p66^{shcA}$ null mice demonstrated reduced serine phosphorylation (serine 307+serine 636-639) of the insulin substrate IRS-1, an hallmark of insulin desensitization.
Conclusions: These findings confirm and extend our observation that $p66^{shcA}$ participates in the regulation of insulin action in an hyperinsulinemic setting, as it occurs in ageing-associated metabolic disorders including obesity and metabolic syndrome. While the possible connection between p66-dependent oxidative stress and insulin resistance needs to be further investigated, our data strongly encourage pharmacological research aimed at blocking the deleterious effect of the $p66^{shcA}$ protein to improve glucose homeostasis and prevent type 2 diabetes.

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Lack of inducible nitric oxide synthase prevents lipid infusion-induced insulin resistance in mice
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Background and aims: The role of inducible nitric oxide synthase (iNOS) on lipid-induced insulin resistance was examined in iNOS knockout (KO) mice and wild-type litter mate using hyperinsulinemic-euglycemic clamp technique.
Materials and methods: Four days before clamp, chronic catheter was inserted into jugular vein and connected to three-way to infuse insulin (15 $pmol \cdot kg^{-1} \cdot min^{-1}$) and 20% glucose on the day of clamp. After overnight fasting, 20% of intralipid and 33 U/ml of heparin were infused for 2 hours before and during clamp in lipid group, and saline was infused in saline group.
Results: Body weight was not different between wild-type and iNOS KO mice but epididymal fat mass was significantly elevated in iNOS KO mice. Glucose infusion rate (GIR) to maintain euglycemia was significantly reduced by lipid infusion in wild-type mice but GIR was not reduced by lipid infusion in iNOS KO mice. Lipid infusion produced whole body insulin resistance in wild-type mice but lack of iNOS prevented development of lipid infusion-induced insulin resistance. Whole body insulin resistance was contributed by 30% decrease in skeletal muscle glucose uptake in wild-type mice, whereas lipid infusion had no effect on glucose uptake of skeletal muscle in iNOS KO mice. Skeletal muscle insulin resistance was accompanied with decrease in glycolysis in lipid-infused wild-type mice. Plasma level of tumor necrosis factor-α was increased by lipid infusion in both iNOS KO and wild type mice. Proinflammatory cytokines in skeletal muscle and adipose tissue were also increased by lipid infusion in both groups. While nitrotyrosine level in skeletal muscle was increased by lipid infusion in wild-type mice, it was significantly lower in lipid-infused iNOS KO mice compared with lipid-infused wild-type mice.
Conclusion: These results suggest that lack of iNOS prevents whole body and skeletal muscle insulin resistance induced by lipid infusion, which may be contributed by reduced nitrosative stress.
Supported by: KOSEF

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Inhibition of PSGL-1-selectin pathway ameliorates obesity-related insulin resistance in db/db mice
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Background and aims: There have been accumulating evidences that inflammation in adipose tissue is involved in the mechanism of obesity-related insulin resistance. Macrophages and proinflammatory cytokines are increased in visceral adipose tissues of obese people and animal models. Infiltration of monocyte/macrophage is mediated by the interaction of cell adhesion molecules expressed on monocytes and endothelial cells. We previously screened the gene expression profiles in adipose tissues from obese mice using DNA microarray and found that P-selectin glycoprotein ligand-1 (PSGL-1) is upregulated in both db/db mice and high-fat diet (HFD) fed mice. PSGL-1 is expressed on both leukocytes and endothelial cells and binds to P-, L- and E-selectin. We found PSGL-1 is expressed on both endothelial cells and macrophages in adipose tissues of obese mice. Furthermore, we demonstrated that PSGL-1 deficient mice fed with HFD revealed decreased macrophage infiltration into adipose tissues, improved adipocyte hypertrophy and insulin resistance as compared with wild type mice fed with HFD. The aim of this study is to identify the inhibitory effect of the PSGL-1-selectin pathway using anti-PSGL-1 monoclonal antibody.
Materials and methods: Anti-PSGL-1 monoclonal antibody or normal rat IgG was administered by intraperitoneal injection to six week-old male db/db mice. An intraperitoneal insulin tolerance test (IPITT) and an intraperitoneal glucose tolerance test (IPGTT) were performed at 7 weeks old. We measured serum lipids, insulin, HbA1c and adipocytokines. We also examined the gene expression of proinflammatory cytokines, chemokines, adhesion molecules, adipocytokines and infiltration of macrophages in epididymal white adipose tissues (WAT). The size of adipocytes was measured and analyzed by morphometry.

Results: There was no significant difference in body weight, weight of epididymal WAT, total cholesterol, free fatty acid and HbA1c. Significant reductions were observed in fasting blood glucose (104.5 vs 138.5 mg/dl) and LDL cholesterol (6.25 vs 10.5mg/dl) in treated group (treated group vs control group, $p<0.05$). The average values of triglyceride and fasting IRI were decreased in treated group as compared with control group. The size of adipocytes in epididymal WAT was also significantly decreased in treated group as compared with control group. Glucose tolerance and insulin sensitivity were significantly improved in treated group in IPITT and in IPGTT. The expressions of MCP-1 and CD68 were decreased in treated group as compared with control group.
Conclusion: The administration with anti-PSGL-1 antibody revealed decreased macrophage infiltration in adipose tissues, improved adipocyte hypertrophy and insulin resistance in db/db mice. These results provide the direct evidence that PSGL-1-selectin pathway promotes the recruitment of macrophages into adipose tissue. PSGL-1 might be a novel target for the prevention of insulin resistance in obesity.
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Decreased serum brain-derived neurotrophic factor concentration in young nonobese subjects with low insulin sensitivity
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Background and aims: Insulin resistance and type 2 diabetes are associated with an increased risk of neurodegenerative diseases. Brain-derived neurotrophic factor (BDNF) regulates neuronal diff0erentiation and synaptic plasticity and its decreased levels are supposed to play a role in the pathogenesis of Alzheimer disease and other disorders. Decreased circulating BDNF levels in obesity and type 2 diabetes were reported, however, it is unclear, whether BDNF might be associated with insulin resistance in young, nonobese population. The aim of the present study was to estimate serum BDNF concentration in apparently healthy, nonobese women divided into subgroups according to their insulin sensitivity.
Materials and methods: We studied 46 young (age: 25.15±5.16 years), apparently healthy, nonobese (BMI: 24.02±2.84 kg x m^{-2}) women with normal glucose tolerance. Anthropometric and biochemical parameters and serum concentrations of BDNF and adiponectin were measured. Insulin sensitivity was estimated with the euglycemic hyperinsulinemic clamp technique. Then, participants were divided into subgroups of high insulin sensitivity (high IS, above median from the clamp study, n=23) and low insulin sensitivity (low IS, below median, n=23).
Results: The difference in BMI and waist circumference between the groups did not reach the level of significance, whereas the percent of body fat was higher in the low IS group ($p=0.024$). We observed decreased serum BDNF concentration in women with low IS ($p=0.001$), which remained significant after adjustment for the difference in the percent of body fat. In the entire study population, serum BDNF was positively related to insulin sensitivity ($r=0.43$, $p=0.003$). In multiple regression analysis, this correlation remained significant after adjustment for other estimated parameters. In the low IS group, relationship between serum BDNF and adiponectin was also observed ($r=0.52$, $p=0.027$).
Conclusions: Our data show that serum BDNF is decreased in young nonobese women with low IS. Thus, early detection and prevention of insulin resistance might be useful in the prevention of neurodegenerative disorders.
Supported by: Poland's Ministry of Science and Higher Education

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Hypothalamic dysfunction in obesity as evaluated by functional magnetic resonance image
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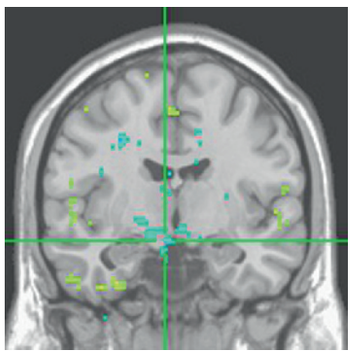
Background and aims: Hypothalamic inflammation and dysfunction have emerged as important factors determining the loss of the coordinated control of caloric intake and energy expenditure in animal models of obesity. Here we use functional magnetic resonance image (fMRI) to explore the hypothesis that obese humans also present some degree of dysfunctional hypothalamic activity.
Materials and methods: Functional images were acquired during a resting state paradigm before and after an oral glucose load. The BOLD signal was recorded for five minutes before and 25 minutes after glucose ingestion, and the Kendall's coefficient of concordance was estimated.
Results: Twelve obese patients undergoing bariatric surgery were submitted to fMRI before surgical procedure and approximately eight months after surgery, when absolute body mass was reduced by 29±4%. Eight age-matched lean controls were evaluated by the same method. Nutritional evaluation at enrolment revealed a mean caloric intake of 5,600±3,400 kCal/day, with high consumption of saturated fat. After surgery, mean caloric intake dropped to 805±350 kCal/day, with substantial reduction of the consumption of saturated fat. Caloric intake of lean subjects was 2,380±850 kCal/day. Reduction of body mass was accompanied by significant reductions of blood insulin and leptin and by the increase of adiponectin. In addition inflammatory markers such as C-reactive protein and blood leukocyte counts were significantly reduced. The comparison of obese patients before surgery with lean controls

revealed a significantly reduced loss of local homogeneity (t-score -8.71, p<0.05) in the hypothalamus (a representative image showing areas with loss of homogeneity in color is depicted in the accompanying figure). After surgery this loss of homogeneity was not so pronounced but still significant (t-score -7.30, p<0.05). Finally, when comparing obese patients before and after surgery a significant change in the loss of homogeneity was detected with a higher score in the post-surgical acquisition (t-score -4.64, p<0.05).
Conclusion: Although the loss of homogeneity in the hypothalamus was still smaller in the post-surgical group than in lean subjects, it is clear that the reduction of body mass was somehow correlated with a modification in the functional pattern of the hypothalamus towards the lean subjects standard. Thus, hypothalamic function, as determined by fMRI differs obese and lean subjects, and massive body mass loss produced by bariatric surgery leads to significant change in this parameter. fMRI may become an attractive, non-invasive method to study central nervous system function in metabolic diseases.



Supported by: Fapesp

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Deep brain stimulation in patients with Parkinson's disease: involvement of local brain regions in systemic glucose metabolism?
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Background and aims: We have previously shown that schizophrenic drug-naïve patients display hepatic insulin resistance, suggesting that central dopamine signaling is involved in the regulation of endogenous glucose production (EGP). As a first step to test this hypothesis in an experimental setting, we studied whether deep brain stimulation (DBS) in the subthalamic nucleus of patients with Parkinson's disease results in a change in basal EGP or hepatic insulin sensitivity.
Materials and methods: We studied 6 patients with Parkinson's disease treated by DBS both in the basal state and during a low-insulinemic euglycemic clamp using stable isotopes with DBS switched on or off. Each subject served as his own control and studies were performed in random assignment. We measured EGP and hepatic insulin sensitivity as well as resting energy expenditure (REE), glucoregulatory hormones and the Unified Parkinson's Disease Rating Scale (UPDRS).
Results: We included 6 men (age 60 [44-65] years and BMI 28.2 [22.6-33.1] kg/m^2). REE was not significantly different between the on and the off-situation. UPDRS was significantly higher when DBS was switched off. There was no significant difference in glucoregulatory hormones in either state. Basal plasma glucose and EGP (after 15 hrs of fasting) did not differ when DBS was switched on or off (EGP on 8.32 ± 0.73 and off 8.22 ± 1.09 μmol/kg·min, p=0.68). Hepatic insulin sensitivity did not significantly change (EGP on 3.15 ± 1.07 and off 2.8 ± 0.91 μmol/kg·min, p=0.36).
Conclusion: Deep brain stimulation of the subthalamic nucleus in patients with Parkinson's disease does not influence basal endogenous glucose production or hepatic insulin sensitivity.

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Reduced hypothalamic insulin receptor expression and insulin-dependent Akt phosphorylation in a type 2 diabetes model associated with a defect in serotonergic system
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Background and aims: Insulin resistance both in the periphery and the brain act synergistically in the induction of metabolic diseases (MD) and co-morbidities. Previous own studies and literature suggest a cross-talk between insulin and the neurotransmitter serotonin (5HT), a regulator of energy and glucose homeostasis, key element in depressive syndromes. In the present study, using a T2D model, the Goto Kakizaki (GK) rat, we focused on hypothalamic and liver insulin signalling and on the impact of 5HT.
Materials and methods: Male Wistar (W) or GK , 8-12 weeks old rats. Insulin receptor (IR) and Phospho-Tyrosine Phosphatase 1B (PTP-1B) protein expression or phosphorylation of Akt (a downstream protein kinase in IR/IRS/PI3K signalling pathway), following ip injection of either insulin (1 U/kg) or dexfenfluramine (stimulator of 5HT, 5 mg/ Kg) 30 min before euthanasia, was assessed by western blot. Endocrine and metabolic parameters were determined with appropriate methods. Statistical significance was set at p<0.05. N= 5-10.
Results: Compared to age matched W, glycemia, insulinemia and leptinemia was increased in GK rats. In W and GK rats, dexfenfluramine increased insulinemia and glycemia but did not alter leptinemia. Glycemia was increased more in GK as compared to W. IR protein expression was lower in GK liver and hypothalamus. In the hypothalamus, insulin injection induced Akt phosphorylation only in W rats. In liver, GK exhibits higher expression of PTP-1B associated to a lower insulin-dependent Akt phosphorylation, as compared to W. Finally, dexfenfluramine stimulated Akt phosphorylation only in the hypothalamus of W rats.
Conclusion: In diabetes, tissue specific alterations in insulin signalling occur within peripheral tissues. Here, were observed important alterations in hypothalamic and hepatic insulin signalling. In spite of inefficient insulin-induced Akt phosphorylation in GK probably due to altered IR and higher expression level of PTP1B, insulin lowered glycaemia, confirming that insulin resistance is not yet totally established in young adult GK. The effect of serotonin on central insulin signalling in W, reported for the first time, extends previous own work shown central insulin-serotonin interaction. The impact of the serotonergic system in GK was altered. In fact, it failed to phosphorylate hypothalamic Akt and increased glycemia in diabetic rats in higher levels than in Wistar, suggesting an exaggerated counter-regulatory effect. This study points out the complexity of insulin-serotonin cross-talk on molecular mechanisms potentially linking depressive disorders and diabetes.
KG and FB, participated equally.

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Sucrose-induced insulin resistance is accompanied by morphologic and functional changes in the adrenal cortex of the rat
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Background and aims: Hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis has been widely described in both human and animals showing insulin resistance (IR). However, a direct effect of the biochemical abnormalities that characterize this syndrome (e.g. elevated plasma glucose, serum insulin and free fatty acid levels, hypertriglicerydemia and oxidative stress) on adrenal function has not been elucidated yet. In this study we assessed the effect of a sucrose-enriched diet (SED) on adrenocortical structure and function (corticosterone secretion) in rats.
Materials and methods: Male Wistar rats were fed a sucrose-enriched diet (SED, drinking water contaning 30% w/v sucrose) up to 12 weeks. Rosiglitazone (4mg/kg, orally and daily) was administered throughout the duration of the sucrose treatment to a group of animals. Protein levels of different isoforms of nitric oxide synthase (NOS), phosphorylated Akt and cyclooxygenase 2 (COX-2) were analyzed by immunoblot while mRNA levels of steroidogenic

acute regulatory protein (StAR) and the macrophage marker F4/80 were assessed by semiquantitative RT-PCR. Steroid levels were determined by RIA. Sudan III staining was performed on adrenocortical slices previously fixed in 4% formaldehyde.
Results: As compared to controls, rats under SED for 7 weeks showed higher fasting plasma glucose (74 ± 5 and 130 ± 6 mg/dl; p<0,001, Mann-Whitney test) and serum triacylglyceride (104 ± 52 vs.604 ± 60 mg/dl; p<0,001) and insulin concentrations (0.99 ± 0.14 vs. 1.97 ± 0.4 ng/ml; p<0,005). An impairment in the insulin signalling pathway was detected at adrenal level as decreased p-Akt protein levels were measured by immunoblot analysis. The adrenal glands were lighter and showed a significant lipidic infiltration, as demonstrated by histochemistry. NOS activity and the expression levels of eNOS, iNOS and COX-2 were increased in the SED group. StAR and F4/80 mRNAs were also elevated. These animals showed significantly elevated basal serum corticosterone levels (6,63 ± 1,14 vs. 9,62 ± 0,84 ng/ml p<0,001) but a lower response to an acute stimulation with 4 UI/kg ACTH i.v., (115,68 ± 34, 03 vs 40,59 ± 25,39 percentage stimulation, p<0,05). On another set of experiments, rosiglitazone, a selective PPARgamma agonist, reverted the nutritionally-induced changes in NOS activity and corticosterone levels and decreased lipid infiltration in the adrenal tissue.
Conclusions: A sucrose enriched diet seems to induce IR at adrenal level after 7 weeks of treatment, generating morphological and functional disturbances that finally could lead to the dysregulation of adrenal steroidogenesis. In this sense, the increase in NOS activity could trigger posttranscriptional modifications of several proteins (nitration, S-nitrosilation etc). Among them, those involved in steroid biosynthesis and its regulation. Both COX-2 and F4/80 could also be related to the chronic inflammatory state linked to IR in several tissues. Finally, some of these effects were prevented by rosiglitazone treatment, suggesting a signal transduction pathway that could be a target for pharmacological interventions designed to ameliorate this adrenal disfunction.
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Glucose metabolism and cognitive dysfunction
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Background and aims: The association between type 2 diabetes and different forms of cognitive impairment is well established. The mechanism behind the association is however still unrevealed. We have recently reported that raised blood glucose levels were associated to impairment in episodic memory, the memory function first affected in the progress to dementia. However, patients with type 2 diabetes have not only elevated levels of blood glucose, but also increased levels of insulin because of insulin resistance. It has been suggested that insulin itself might have a negative effect on cognitive function and memory. Diabetes is associated with a long standing hyperglycaemia but also with hypertension and hyperlipideima, leading to micro and macro vascular disease. Thus, our aim was to study whether insulin affects episodic memory independently of glucose in a nondiabetic adult population.
Materials and methods: We linked and matched two large population based data sets in Sweden, the Betula study and the Västerbotten Intervention Program. We identified 364 (F/M 207/157, mean age 50.5 ±8.0 years) nondiabetic subjects, free from dementia, who had participated in the two surveys within six months. The memory test included testing of episodic memory. We transformed the results using the mean values and standard deviation from the youngest age group to compute a composite *z*-score (subjects' value minus mean score in the 40-year-old group divided by SD). Fasting plasma insulin (FPI) and glucose (FPG) were analyzed with standard methods.
Results: Women had higher levels of episodic memory (mean z-score -0.06, SD 0.54) compared to men (mean z-score -0.36, SD 0.51, p<0.001). Given the sex difference in the outcome variable we stratified for sex. In a univariate linear regression both FPG (B -0.274, SE 0.068, Beta -0.271, p<0.001) and FPI (B -0.389, SE 0.131, Beta -0.204, p=0.003) were significantly associated with episodic memory in women but not in men. FPG, but not FPI, remained significantly associated with episodic memory after adjustment for hypertension, total P-cholesterol, bodymass index, educational level, depression, smoking and cardiovascular disease (FPG: B -0.218, SE 0.070, Beta -0.220, p=0.002; FPI: B -0.232, SE 0.149, Beta -0.127, p=n.s.), when FPG and FPI were analyzed separately. Entering both FPG and FPI into the regression model did not attenuate the association between FPG and episodic memory (FPG: B -0.204, SE 0.071, Beta -0.206, p=0.005).
Conclusion: We conclude that an increase in plasma glucose, but not plasma insulin, is associated with impairment in episodic memory in women. This could be explained by a negative effect on the hippocampus caused by raised plasma glucose levels.
Supported by: The Västerbotten County Council

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Cognitive function in older adults with type 2 diabetes
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Background and aims: Several studies have reported increased risk of Cognitive Dysfunction (CD) and dementia in people with Type 2 Diabetes (DM2). The outcome of these research efforts indicate that several causes may affect CD, include many pathophysiologic mechanisms and risk factors. We investigated DM2 as a risk factor for CD when compared with control group. Describe the frequency of DC in patients with DM2 and its relationship to education, income, physical activity, alcohol consumption, cardiovascular risk factors and depression.
Materials and methods: Cognitive function was assessed using the test Mini Mental State Examination (MMSE) and defined by scores (normal 28-30, mild cognitive dysfunction (MCD) between 27 to 24, and severe cognitive dysfunction (SCD) 9 points and pathological 65 years old and control group. The study was carried out by 17 Argentinean doctors specialized in Diabetes from the provinces of Buenos Aires, Salta and Buenos Aires City (Argentina). The evaluation included the following items: anthropometric measures, laboratory, level of education, life style and income. Chi2, Wilcoxon test and Multiple Logistic Regression were the tools used for the statistical analysis.
Results: 748 patients were included: DM2 (DMG) n=433 and control group (CG) n=315. Mean age=71.83 ± 5.58 in DMG and 71.79 ± 5.51 years in CG (p>0.92). In DMG the duration of diabetes 11.78 ± 9.17 years, the average HbA1c was 7.15 ± 2.73. The alcohol consumption was 48.73% in the DMG and 46.98% in CG. Education under 7 years was 40.8% in DMG vs. 35.67% in CG (p=0.39). The monthly income < 300 euros was 27.48% in DMG and 27.94% in CG (p=0.87). Depression was present in 18.2% of DMG and 12.4% of CG. SCD was present in 21.3% of DMG and 14.6% of CG and MCD in 35.3% of DMG and 31.7% of CG (p<0.0018). In women, diabetes acted as a risk factor for cognitive dysfunction (crude OR=2.13, 95% CI=1.46-3.11, p<0.0001). In men the mentioned above relation was not statistically significant (crude OR=1.14, 95% CI=0.71-1.85; p=0.57). In multivariate analysis, cognitive dysfunction in women was related with the presence of DM2 (adjusted OR=1.91; CI=1.26-2.90; p=0.002), low education level (OR=3.96; CI=2.55-6.13, p<0.0001), low-income (OR=2.06; CI=1.29-3.26; p=0.002), depression (OR=1.83; CI=1.08-3.04, p=0.026) and alcohol use (OR=1.57M; CI=1.01-1.45; p=0.04). In multivariate analysis, independent predictors of cognitive decline in men were low education (OR=4.9; CI=2.28-6.88; p<0.0001), low-income (OR=2.33; CI=1.14-4.58; p=0.01) and age (OR=2.25; CI=1.08-3.20; p=0.02).
Conclusion: The outcomes of our study indicate that DM2 in women increased the risk of CD. The low education level, low income, presence of depression and alcohol consumption were independent risk predictors. DM2 in men was not a predictor of CD in this sample. Low education, low income, and age were independent risk predictors of CD in men.
Supported by: Argentine Society of Diabetes

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Brain volume changes in type 1 diabetes mellitus patients with microangiopathy are related to poorer cognitive functioning
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Background and aims: Type 1 diabetes (T1DM) has been associated with lower amounts of brain volume as compared to matched healthy controls.

Furthermore, T1DM is associated with cognitive impairment, most pronounced in patients with microangiopathy. Here, we hypothesized that brain volume loss would be most marked in T1DM patients with microangiopathy and would be associated with impaired cognitive functions. Using magnetic-resonance imaging (MRI), we quantified grey and white matter volume and total brain volume and performed a neuropsychological assessment in 155 T1DM patients with and without microangiopathy and matched healthy controls.
Materials and methods: Fifty-one T1DM patients with and 53 without microangiopathy, and 51 matched healthy controls underwent a detailed neuropsychological assessment including the domains of general cognitive ability, memory, information processing speed, executive functions, attention, motor and psychomotor speed and a MRI-scan. This MRI-scan consisted of 10 different sequences to detect differences in cerebral structure and function among which was a T1 Magnetization Prepared Rapid Gradient Echo (MP-RAGE) for the estimation of both grey and white matter volume and total brain volume. Volumes were estimated using the Structural Image Evaluation, using Normalisation, of Atrophy (SIENAX) tool in the FMRIB Software Library (FSL4.1). This tool enables reliable estimation of brain volume by controlling for differences in brain size.
Results: Both grey matter volume and total brain volume were significantly decreased in T1DM patients with microangiopathy, compared to T1DM patients without microangiopathy and healthy controls (both $P < 0.05$). However, these differences were lost when controlling for age. In T1DM patients with microangiopathy a moderate positive correlations between motor speed and both white matter volume and total brain volume was found. T1DM patients without microangiopathy demonstrated a small positive correlation between motor speed and white matter volume ($P < 0.05$). In controls no correlations were found between brain volumes and cognitive function domains.
Conclusion: After correction of age, no differences in brain volume could be detected between T1DM patients with and without microangiopathy and healthy controls. Nevertheless, in T1DM patients positive correlations of MRI-measured brain volumes and motor speed could be demonstrated. These data may suggest that loss of brain volume is associated with poorer cognitive performance in this domain, although no causal relationships can be established. Longitudinal studies are warranted to confirm and expand these findings, to further detail the underlying mechanisms and to define their impact on patients, in terms of clinical consequences and quality of life.
Supported by: Dutch Diabetes Research Foundation

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Cerebral functioning is associated with carotid intima media thickness in uncomplicated type 1 diabetes mellitus

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Background and aims: Measures of subclinical atherosclerosis, including carotid intima media thickness (cIMT), have shown to negatively affect brain function and structure in the general population. In type 1 diabetes (T1DM), an increased cIMT has been reported, but it is unknown whether cIMT is associated with cerebral function and activity in these patients and whether this association is affected by the additional presence of microvascular complications.
Materials and methods: We investigated 51 T1DM patients with proliferative diabetic retinopathy or other microvascular complications, as a marker of microangiopathy (DRP), 54 without microangiopathy (non-DRP), and 51 gender-matched controls. We measured cIMT using ultrasound and neuropsychological functions like memory, information processing speed, executive functions, attention, motor and psychomotor speed and general cognitive ability. Functional brain connectivity, an estimate of cerebral communication, was assessed using magnetoencephalography. Linear regression was used to determine the association between cIMT, cognitive functions and functional connectivity.
Results: In the non-DRP group, but not in DRP patients, cIMT was negatively associated with general cognitive ability, information processing speed and attention (all $P<0.05$). Also, in non-DRP patients a positive association of cIMT and functional connectivity for the delta-band, representing communication within both hemispheres and a negative association for the upper gamma-band, regarding interhemispheric communication were found (both $P<0.05$). Except for information processing speed all associations were independent of age, gender, diabetes duration and onset age.
Conclusion: In uncomplicated T1DM patients, but not in patients with microangiopathy, cIMT was inversely associated with cognitive functioning and related to aspects of functional connectivity, previously linked to cerebral pathology and changes in cognitive functions. Taken together, these findings suggest that, in T1DM patients without microangiopathy, subclinical atherosclerosis may exert a negative effect on the brain, whereas in T1DM with microangiopathy the impact of cIMT seems subordinate to other factors.
Supported by: Dutch Diabetes Research Foundation

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Comprehensive neuropsychological assessment of patients with longstanding type 1 diabetes mellitus with and without microangiopathy

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Background and aims: Type 1 diabetes (T1DM) is associated with mild cognitive decrements, and more so in the presence of proliferative retinopathy (DRP) as was shown in a small study comparing DRP patients with patients without DRP (non-DRP) and controls. In the present study, we hypothesized that T1DM patients with DRP would show cognitive decrements compared to T1DM patients without DRP and other microvascular complications and controls.
Materials and methods: Fifty-one DRP patients, 54 non-DRP patients and 51 controls underwent a comprehensive neuropsychological assessment, covering the domains general cognitive ability, memory, information processing speed, executive functions, attention, motor and psychomotor speed. Prior to assessment, blood glucose level of T1DM patients had to be in the range of 4-15 mmol/l and hypoglycaemic events 24 hours prior to assessment resulted in rescheduling the assessment. MANCOVA corrected for age, depressive symptoms and multiple comparisons was used to determine group differences and regression analysis to identify determinants of cognitive decrements.
Results: T1DM patients as one group compared to controls showed decrements in general cognitive ability, memory, information processing speed, motor and psychomotor speed (all $P\neg<0.05$). Effect sizes ranged from 0.3 - 0.6. Gender, proliferative DRP, blood pressure and blood glucose level were independent predictors of these decrements. When separating both T1DM patient groups, DRP patients were found to be oldest, reported most depressive symptoms, had longest disease duration and earliest age of diabetes onset. Both non-DRP and DRP patients showed reduced information processing speed compared to controls, with DRP patients showing the largest deterioration. DRP patients also showed decrements in memory, motor and psychomotor speed compared to controls and in attention and general cognitive ability compared to non-DRP and controls (all $P\neg<0.05$).
Conclusion: In this large sample, we showed that T1DM patients as compared to controls are susceptible to cognitive decrements. Furthermore, patients with DRP seem to be at the highest risk to develop cognitive decrements. Interestingly, patients without microangiopathy appear also susceptible to loss of processing speed, suggesting that selective cognitive functions are affected before microangiopathy becomes manifest.
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PS 63 Novel targets in insulin resistance

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Apical sodium-dependent bile transport inhibitors is identified as potential antidiabetic agents: parallels with metformin

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Background and aims: The presence of bile acids (BA's) in the distal gut powerfully stimulates secretion of GLP-1 and other peptides from enteroendocrine L-cells. An effect of metformin is the inhibition of bile acid reuptake, which is mediated via apical sodium-dependent bile transporters (ASBT) in the ileum. Especially in the presence of dipeptidyl peptidase-IV inhibition (DPP4i), metformin stimulates secretion of L-cell products, including GLP-1.
Materials and methods: To probe the hypothesis that metformin's therapeutic effects are mediated via ASBT inhibition, we compared the dose-dependence of secretion of active GLP-1 in fasted normal rats pre-treated with a DPP4i (sitagliptin) and orally administered metformin (0, 30, 100, 300 mg/kg) or a non-absorbable ASBTi (SC-435; doses 0, 3, 30, 100 mg/kg). In db/db mice we tested the effects of orally administered metformin (0, 3, 30, 100, 300 mg/kg; n=5-12/group) and SC-435 (0, 3, 30, 100 mg/kg; n=9-12/group) on glucose level and 48 hour body weight.
Results: SC-435 dose-dependently increased 5-hour integrated GLP-1 concentrations 2.5 to 3.2 fold (-/+ DPP4i, respectively) vs vehicle, and metformin evoked 3.3 to 4.3 fold increases. SC-435 was ~1.6 fold more potent than metformin. Peak [GLP-1] of 30-36 pM observed 4-5 hours after metformin or SC435 in the present studies may promote antidiabetic and weight-loss effects otherwise only attainable with injected GLP-1 agonists. In db/db mice, a 7.9 mg/dL reduction in 24-hour plasma glucose invoked by 30 mg/kg valine-pyrolidide (a DPP4i) was amplified up to 3.8 fold by the addition of SC-435 This exceeded the antidiabetic effect of metformin in this model. Both SC-435 and metformin (+DPP4i) dose-dependently reduced body weight (by up to 5.2%, 4.3% respectively) over 48 hours in db/db mice, SC 435 being 10.5 fold more potent.
Conclusion: In summary, the parallel behavior of metformin and a non-resorbable bile salt transport inhibitor to stimulate GLP-1 secretion, to lower plasma glucose, to lower body weight, and to elevate BA's in the distal bowel, are consistent with their actions being via indirect L-cell stimulation with BA's. These studies further identify ASBTi's, including those without systemic exposure, as a potentially new class of oral antidiabetic antiobesity agent.

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Effects of angiotensin II on receptor mediated insulin transcytosis in bovine aortic endothelial cells

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Background and aims: Angiotensin II(ATII) is involved in the pathogenesis of hypertension and increases insulin resistance. It is known that the main role of insulin receptor in endothelial cells is to deliver insulin to target tissue by a series of process - binding of insulin on insulin receptor, internalization of insulin-insulin receptor complex and releasing of insulin around target tissue - called receptor mediated transcytosis. We investigated the effects of ATII on receptor mediated insulin transcytosis in endothelial cell.
Materials and methods: After treating with {insulin alone}, {insulin and ATII}, {insulin, ATII and angiotensin receptor blocker(ARB)} on bovine aortic endothelial cells, we observed the change of the total amount of insulin receptor, the amount of insulin receptor on membrane and in cytosol, binding of insulin on insulin receptor and internalization of insulin-insulin receptor complex, time dependently.
Results: 1) Insulin increased the total amounts of insulin receptor, binding of insulin on insulin receptor and internalization of insulin-insulin receptor complex. 2) ATII decreased the total amount of insulin receptor, and it decreased the binding of insulin on insulin receptor up to 70 %. 3) After treating with ATII, the amount of insulin receptor on cell membrane was decreased but the amount of insulin receptor in cytosol was increased, time dependently. 4) ATII decreased the internalization of insulin-insulin receptor complex, but the difference is imperceptible. 5) ARB improved the ATII- induced reduction of binding of insulin on insulin receptor up to 30 %.
Conclusion: It seems that ATII inhibits the receptor mediated insulin transcytosis in endothelial cell by reducing the binding of insulin on insulin receptor, and ARB improves this inhibitory effect. We think that this inhibitory effect is due to reduction of the amount of insulin receptor on cell membrane by reduction of the expression of insulin receptor and increase of the translocation of insulin receptor from cell membrane to cytosol. As a result, the delivery of insulin around target tissue is decreased, and this may explain partially insulin resistance by ATII.

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Taurine improves hepatic insulin resistance induced by free fatty acid through inhibiting c-Jun NH2-terminal kinase 1(JNK1) pathway and improving insulin signalling

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Background and aims: It is well established that elevated circulating free fatty acids (FFA) can induce hepatic insulin resistance, which is the primary contributor to increased hepatic glucose production and overt hyperglycemia in type 2 diabetes. However, the exact mechanism by which FFAs cause insulin resistance in the liver is not completely known, but may involve oxidative stress. Intralipid plus heparin (IH) infusion is a standard nontoxic method to elevate FFA levels in vivo. Therefore, we established an in vivo model of insulin resistance by prolonged (48h) IH infusion in wistar rats. And co-infusion with taurine (an antioxidant) was designed to investigate the effects of taurine on hepatic insulin resistance, and to explore the potential mechanism of IH-induced hepatic insulin resistance in prolonged lipid infusion model.
Materials and methods: Cannulated rats (n=12-14/group) were infused for 48h intravenously with saline(SAL), or intralipid plus heparin (IH), or IH plus taurine (IHT), or taurine (TAU). Half of each group rats were randomly assigned to either the basal group or a clamp group. During the last 2h of the 48h infusion, hyperinsulinemic-euglycemic clamps with [6-^{3}H] glucose infusion (20μCi bolus followed by constant infusion at 0.4 μCi/min) were performed to assess hepatic insulin sensitivity. Oxidative stress measured as the concentration of 8-Iso-Prostaglandin F2a (8-iso-PGF2α) in liver was analyzed using anenzyme-linked immunoassay (ELISA) commercial kit. We evaluated JNK1 activation, insulin-stimulated IRS-1 tyrosine phosphorylation and insulin-stimulated IRS-2 tyrosine phosphorylation in the liver by immunoprecipitation and immunoblotting.
Results: The results showed during hyperinsulinemic-euglycemic clamp, IH infusion induced hepatic insulin resistance as indicated by elevation of HGP compared with SAL. Taurine prevented the elevation of HGP induced by IH infusion (HGP in umol/kg.min, SAL: 25±4 , IH: 71±6 , IHT: 30±7 , TAU: 26±4 , $p<0.05$ IH vs. other treatment groups). This indicates that IH-induced hepatic insulin resistance was abolished with taurine co-infusion. Furthermore, taurine co-infusion reversed IH-induced 1) increase in 8-iso-PGF2α, 2) increase in JNK1 activity, 3) decrease in insulin-stimulated tyrosine phosphorylation of IRS-2 in liver. Insulin-stimulated tyrosine phosphorylation of IRS-1 in liver did not differ among the four groups in this study.
Conclusion: In summary, prolonged IH-infusion induced the impairment of hepatic insulin sensitivity probably by activating JNK1 pathway and reducing the insulin-stimulated tyrosine phosphorylation of IRS-2 in liver. Taurine, as an antioxidant, prevented hepatic insulin resistance might also associated with inhibiting JNK1 and increasing insulin-stimulated tyrosine phosphorylation of IRS-2 in vivo.
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Probiotic Bifidobacterium lactis 420 reverses diabetic status in mice under high-fat diet by reducing plasma endotoxin and tissue inflammation

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Background and aims: It has been proposed that alterations in the intestinal microbiota may be causally involved in high-fat diet (HFD)-induced meta-

bolic diseases. Therefore, an efficient probiotic treatment should modifies the deleterious impact of the fat-enriched diet on the occurrence of diabetes.
Materials and methods: We here daily treated insulin resistant HFD mice with 10-8 to 10-10 Bifidobacterium lactis 420 daily for 6 weeks.
Results: The probiotic treatment dose dependently reduced glucose intolerance. The treatment with 109cells daily reduced the impact of HFD on body fat mass, mesenteric adipose tissue mass, fasted hyperinsulinemia, and insulin resistance as assessed by the euglycemic clamp technique. Conversely, fed insulin secretion was improved. This improved glucose metabolism was associated with a lowering of plasma LPS concentration i.e. metabolic endotoxemia. Similarly, liver and adipose tissue cytokine mRNA expression (Il6, TNFa, IL1, PAI-1) were reduced. Interestingly, we detected bacteria in the mesenteric adipose depot of HFD mice when compared with normal chow whereas the subcutaneous fat was mostly unaffected. Among these bacteria, the Enterobacteriaceae (LPS containing bacteria) were mainly increased. The probiotic treatment reduced this enrichment. We then explored that a change in intestinal epithelial cells permeability could be at the origin of the translocation of intestinal bacteria towards the adipose tissue in response to a HFD. Our data show that the probiotic extract or supernatant regulates the transepithelial electrical resistance in vitro on Caco2 cells.
Conclusion: Altogether our data suggest that Bifidobacterium lactis 420 treatment improved glucose metabolism of HFD-induced diabetic mice by reducing intestinal bacterial translocation, metabolic endotoxemia, and adipose tissue inflammation.

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Discovery of a compound with potent efficacy on the activation of AMPK and treatment of type 2 diabetes
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Background and aims: Glucose uptake in skeletal muscle plays a key role in the maintaining glucose homeostasis. YL01 was discovered from our compound collections which could increase glucose uptake in L6 myotubes. In the present study, the *in vivo* anti-diabetic effect of YL01 was evaluated and its possible mechanisms were explored.
Materials and methods: D-2-[^{3}H]deoxyglucose uptake and [^{3}H] palmitate oxidation assay were performed to examine the effects of YL01 on the glucose uptake and free fatty acid oxidation in L6 myotubes. Activation of YL01 on the AMPK and insulin signaling pathway was investigated by western blot analysis. For *in vivo* study, YL01 was administered orally to *ob/ob* mice at the dose of 200mg/kg for 23 days. Metformin was used as positive control.
Results: YL01 dose dependently enhanced the glucose uptake and free fatty acid oxidation in L6 myotubes. Incubation of L6 myotubes with 3μM YL01 resulted in a 1.8 and 1.5 fold increase in basal glucose uptake and free fatty acid oxidation, respectively. YL01 showed no effect on Akt phosphorylation, but significantly increased AMPK and ACC phosphorylation in L6 myotubes. However, YL01 could not directly activate the recombinant AMPK kinase. The YL01 induced glucose uptake and fatty acid oxidation could be fully blocked by the pretreatment with compound C, an AMPK inhibitor. Oral administration of YL01 significantly reduced both of the non-fasting and fasting blood glucose levels and improved the impaired glucose tolerance of *ob/ob* mice. Moreover, the HbA1c levels in *ob/ob* mice also showed a decrease tendency after 23 days treatment with YL01 although it didn't reach statistic significance.
Conclusion: YL01 exerts antidiabetic effect on ob/ob mice, which suggested that it might be a therapeutic candidate for the treatment of T2DM and metabolic syndrome. Indirect activation of AMPK might be one of the possible mechanisms of this effect.

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AT1-receptor blockade and insulin sensitivity in hypertensive subjects: the role of capillary recruitment
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Background and aims: Blocking the renin-angiotensin system (RAS) improves insulin sensitivity in hypertensive subjects. However, the underlying mechanisms are undefined. An effect of insulin that is crucial for stimulating glucose uptake is the ability of insulin to regulate its own delivery, and that of glucose, to muscle cells via recruitment of the microvasculature. This study was designed to investigate the effect of acute angiotensin II AT1-receptor blockade (ARB) on insulin-mediated microvascular function and insulin-mediated glucose uptake in hypertensive subjects.
Materials and methods: A randomised, double-blind placebo-controlled trial was performed in 15 untreated mildly hypertensive subjects (BMI 26.9±2 kg/m^2; BP 150/92 mmHg), to examine the effects of acute ARB treatment (irbesartan, 600mg, oral single dose) or Ca^{2+}-blockade (felodipine, 10mg idem) as a pressor control on insulin-induced microvascular function and on insulin-mediated whole body glucose uptake (WBGU, mg/kg/min) during a hyperinsulinaemic euglycaemic clamp (50mU/kg/h). Effects of irbesartan and felodipine were compared to placebo. Skin capillary density (n/mm^2) and capillary recruitment (peak n/mm^2 during post-occlusive reactive hyperaemia, PRH) were measured with capillaroscopy. All subjects were tested on a low sodium diet (100 mmol/day).
Results: Compared to the basal state, hyperinsulinaemia increased baseline capillary density (56.8±7.1 vs. 60.2±8.3 n/mm^2, P<0.02). Relative to placebo, irbesartan, but not felodipine, increased insulin-induced capillary density (Δ cap density (median (interquartile range) +3.5 (-1.3 - +5.0) n/mm^2, P<0.02). Insulin-induced capillary recruitment was not altered by either treatment. Neither irbesartan nor felodipine enhanced WBGU.
Conclusion: Our data demonstrate that acute AT1-receptor blockade augments insulin-induced capillary density in mildly hypertensive subjects. Although glucose uptake did not increase significantly, the increased insulin-induced microvascular function found with ARB might point to improved insulin and glucose delivery as the underlying mechanism for the improved insulin sensitivity with longterm ARB treatment.

PS 64 Other hormones and endogenous factors

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Ciliary neurotrophic factor increases plasmatic insulin half-life and improves metabolic profile in a non-insulin resistance type 2 diabetes mellitus model

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Background and aims: Type 2 diabetes mellitus (DM2) is characterized by impaired insulin sensitivity and secretion, leading to hyperglycaemia. CNTF is a cytokine that improves metabolic profile in obesity-induced and insulin resistant DM2 models, allegedly through an increase in insulin sensitivity. Besides that, CNTF promotes in vitro pancreatic islet survival. Given that, we decided to evaluate the role of functional beta cell mass maintenance by CNTF in a non-insulin resistant DM2 model. Insulin clearance is an important process that plays a role in controlling insulin action, usually evaluated as plasmatic insulin half-life. Abnormalities in this process are involved in many metabolic disorders, particularly in DM2.
Materials and methods: Neonate Swiss mice received intra-peritoneal injection of Citrate buffer (CTL), CNTF 0,1mg/Kg (CNTF), Alloxan 250mg/Kg (ALOX) or a combination of both (CNTF+ALOX). We performed an intraperitonal glucose tolerance test (ipGTT) in p26 and an intraperitonal insulin tolerance test (ipITT) in p28. Plasma glycaemia was assessed by a Roche Accu-Chek II Glucometer. Plasma insulin was assessed by Radioimmunoassay (RIA). Data are expressed as mean +/- SEM, and p<0,05.
Results: Alloxan-treated mice were hyperglycaemic and glucose intolerant, indicating that they are diabetic (DM2). Nevertheless glycaemia decrease after ipITT was similar to CTL, therefore they were not insulin resistance. CNTF+ALOX mice had lower fasting and similar fed glycaemia than CTL. Besides the glycaemia decreased after ipITT was similar to CTL. CNTF increased plasmatic insulin half-life in both CNTF and CNTF+ALOX groups (Figs.3-4), despite the fact that its impairs pancreatic islet glucose-stimulated insulin secretion.
Conclusion: The results indicate that CNTF improved metabolic profile in non-insulin resistant DM2 model, similarly to other insulin resistant models, such as obesity, suggesting that CNTF protective effects might involve mechanisms other than just increased insulin sensitivity, specially by increased plasmatic insulin half-life, and supposedly also through functional beta cell mass maintenance.

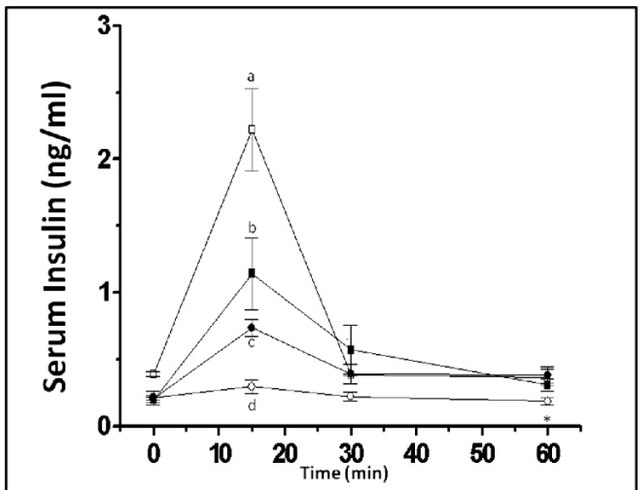

Fig.3. Serum Insulin during whole-body glucose tolerant test at p26. Blood insulin was measured from fasted mice tail at 0, 15, 30 and 60 min after intraperitoneal injection of 2 g/Kg glucose. N = 4 and *p<0,05 for difference from Control (CTL). Different letters represents statistically significant difference.

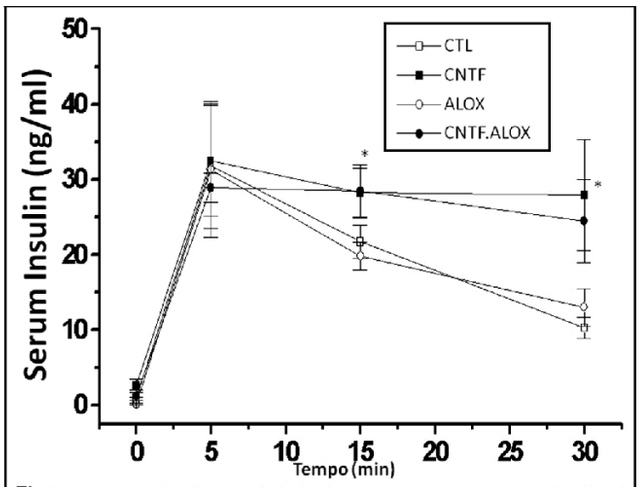

Fig.4. Serum Insulin during whole-body insulin tolerant test at p28. Blood insulin was measured from fed mice tail at 0, 5, 15 and 30 min after intraperitoneal injection of 1,75 UI/Kg insulin. N = 4 and *p<0,05 for difference from Control (CTL).

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Direct effects of FGF 21 on glucose uptake in human skeletal muscle: implications for type 2 diabetes and obesity

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Fibroblast growth factor (FGF)-21, a novel member of the FGF family, plays a role in a variety of endocrine functions, including the regulation of glucose and lipid metabolism. We assessed serum levels of FGF-21 and skeletal muscle mRNA in normal glucose tolerant (NGT; n=40) type 2 diabetic patients (n=40). We also determined whether FGF-21 has a direct effect on glucose metabolism in cultured myotubes from NGT subjects (n=8) and adult extensor digitorum longus (EDL) skeletal muscle. Serum FGF-21 levels were increased 20% in type 2 diabetic versus NGT subjects, whereas skeletal muscle mRNA expression was unaltered. Fasting insulin, HOMA-IR, waist circumference and BMI were significantly correlated with serum fasting FGF-21 levels in type 2 diabetic, but not NGT subjects (p<0.01). Serum FGF-21 concentrations were significantly greater in the type 2 diabetic patients in the highest tertile of fasting insulin (p<0.05) and BMI (p<0.05). Stepwise regression analysis further identified BMI as the strongest independent variable that positively correlated with FGF-21 levels. FGF-21 exposure increased basal and insulin-stimulated glucose uptake in primary human myotubes, coincident with increased GLUT1 mRNA. In isolated EDL muscle, FGF-21 potentiated insulin-stimulated glucose transport, without altering phosphorylation of insulin or AMPK signaling. In conclusion plasma FGF-21 is increased in type 2 diabetic patients, and positively correlated with fasting insulin and BMI. Moreover, FGF-21 has a direct effect on skeletal muscle glucose uptake.
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Characterisation of FGF19, FGF21, and FGF23 stimulated FGFR1/4 activation

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Background and aims: The members of the FGF19-family including FGF19, FGF21, and FGF23 play a distinct role from the other FGFs based on their endocrine action and the necessity of a co-receptor. These proteins exert a wide

variety of metabolic activities like the regulation of bile acid, carbohydrate and lipid metabolism as well as phosphate, calcium and vitamin D homeostasis via binding to and activation of FGF receptors (FGFR) in presence of α-Klotho (KL) or β-Klotho (KLB). Aim of the present study was to characterize the activation of FGFR1 and FGFR4 in presence or absence of KL or KLB by FGF19, FGF21, and FGF23.

Materials and methods: The FGF induced FGFR autophosphorylation was measured via in-cell western (ICW) in CHO cells overexpressing either human FGFR1c short or long form, or FGFR4 in presence or absence of KL or KLB. ERK phosphorylation as a downstream signalling readout for FGFR activation was analysed by ICW in human primary visceral adipocytes. Time dependent *in vivo* signalling of FGF21 in WAT and liver of C57bl6/J mice was analysed using ELISA after s.c. injection of 0.6 mg/kg FGF21.

Results: We established a specific and highly sensitive ICW assay for direct analysis of the FGF induced FGFR autophosphorylation in CHO cells overexpressing different human FGFR ± KL/KLB. EC_{50} values were obtained from dose-response curves and are summarized in table 1. In these CHO cells FGF1 and FGF2 activated either FGFR1 or -4 independent of KL/KLB. FGF19 activated only FGFR4 in presence of KL or KLB. In contrast FGF21 activated the long and short form of FGFR1c efficiently only in complex with KLB. FGF21 was also able to stimulate FGFR4-KLB but was 4 times less potent than FGF19. FGF23 needed the co-receptor KL for an efficient activation of FGFR1 and -4. The analysis of ERK phosphorylation in human primary visceral adipocytes which express mainly FGFR1 and KLB demonstrated that in addition to FGF2 only FGF21 was able to activate FGFR signalling. After s.c. injection of FGF21 in mice a comparable ERK activation was found in fat tissue. No ERK activation was detectable in liver where predominantly FGFR4 is expressed. Contrary to this in WAT FGF21 stimulated a fast increase, reaching a maximum after 30-60 minutes. The decreasing signal was detectable even up to 8 hours.

Conclusion: Using cell lines overexpressing FGFR and co-receptors we could demonstrate that all members of the FGF19-family require the presence of KL or KLB for an efficient signalling through a particular FGFR isoform, e.g. FGF21 only activates FGF signalling in adipocytes that primarily express FGFR1, but not FGF19 or FGF23. We conclude that the expression of KLB and KL, in combination with particular FGFR isoforms, determines the tissue-specific metabolic activities of the FGF19-family.

Summarized in vitro data for FGF induced FGFR phosphorylation in CHO cells & in human adipocytes

Cell line	CHO R1cS	CHO R1cS+KL	CHO R1cS+KLB	CHO R1cL+KLB	CHO R4	CHO R4+KL	CHO R4+KLB	human visceral Adipocytes
	(nmol/L)	**(nmol/L)**	**(nmol/L)**	**(nmol/L)**	**(nmol/L)**	**(nmol/L)**	**(nmol/L)**	**(nmol/L)**
FGF1	7.6±4.2	-	0.8±0.2	-	11.2±9.0	-	13.6±7.4	-
FGF2	1.4±0.2	2.2±0.8	0.9±0.2	1.6±0.5	15.6±10.4	8.0±3.0	19.0±11.2	0.04±0.007
FGF19	>250	>250	>250	>250	>250	63±14.1	54±5.9	>250
FGF21	>250	>250	3.1±0.3	12.8±5.9	>250	>250	213±60	0.81±0.19
FGF23	>250	1.7±0.4	>250	>250	>250	12.1±5.3	>250	>250

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Effects of short-term continuous subcutaneous insulin infusion on fasting plasma Vaspin levels in patients with new-onset type 2 diabetes mellitus

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Background and aims: Visceral adipose tissue-derived serine protease inhibitor (Vaspin) has recently been characterized as an insulin-sensitizing adopokine. However, the roles of this factor in humans remain unknown. This study was aimed to investigate the effects of short-term continuous subcutaneous insulin infusion (CSII) on plasma vaspin levels in patients with severe newly diagnosed type 2 diabetes.

Materials and methods: Thirty patients with severe newly diagnosed type 2 diabetes (T2DM), 37 subjects with impaired glucose regulation (IGT) and 38 sex-, age- and BMI-matched normal controls (NGT) participated in the study. T2DM group was treated with CSII for 2 weeks. Euglycemic-hyperinsulinemic clamps (EHC) were performed in 16 subjects of T2DM group. Plasma vaspin concentrations were measured with a commercial ELISA kit. The relationship between plasma vaspin levels and metabolic parameters was also analyzed.

Results: Fasting plasma vaspin levels were higher in T2DM than in and IGT and NGT groups (1.83+/-0.55 *vs.* 0.43+/-0.21 *vs.* 0.56 +/-0.26microg/L, $P<0.05$), but there was no difference between IGT and NGT groups. Fasting plasma vaspin concentrations were decreased significantly in T2DM group after two-week CSII treatment (1.83+/-0.55 *vs.* 1.19+/-0.57microg/L, $P<0.05$) accompany with significant amelioration of insulin sensitivity and glucose control. Changes in circulating vaspin concentrations were correlated positively with those of insulin sensitivity.

Conclusion: In T2DM patients, plasma vaspin levels are elevated, but significantly decreased after CSII treatment. These data suggest that vaspin play may a role in insulin sensitivity of diabetic humans.

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Omentin, a novel visceral fat depot-specific secretory protein, enhances insulin-stimulated glucose uptake, in peripheral monocytes, in patients with type 2 diabetes

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Background and aims: Omentin is an adipocytokine, selectively expressed and secreted from visceral adipose tissue. Its biological action is not clear, but it is speculated that it has a beneficial effect on insulin action, since it has been shown to increase insulin signal transduction and to enhance insulin-stimulated glucose transport. Omentin plasma levels have been inversely correlated with indicators of metabolic risk, such as body mass index and HOMA. The present study investigates the effect of omentin on glucose uptake in peripheral monocytes from patients with type 2 diabetes (DM) and normoglyceamic subjects (NG).

Materials and methods: Blood (20ml) was withdrawn from 10 treatment-naive patients with type 2 diabetes (BMI 23±2kg/m^2, age 52±5years), and 8 healthy volunteers (BMI 22±2kg/m^2, age 50±6years). Circulating monocytes were incubated for 1 hour with insulin (0, 25 and 100mU/l) or/and omentin (100, 300ng/ml) to determine the abundance of GLUT4 on the plasma membrane. Cells were stained with antiGLUT4 antisera. Glucose uptake was assayed with the addition of insulin and/or omentin and the fluorescent analogue of glucose 6-NBDG and was monitored until plateau was reached. The abundance of surface GLUT4 and the glucose uptake were studied by flow cytometry. Statistical analysis of glucose uptake in response to omentin, insulin and their combination was performed by repeated-measures ANOVA. The comparison between the increments of surface GLUT4 from baseline (absence of insulin or omentin) to maximal hormonal challenge was carried out by paired t-test. Comparisons between DM and NG were performed using unpaired t-test.

Results: In monocytes from NG, insulin increased glucose uptake in a dose-dependent manner ($P<0.001$), as well as the recruitment of GLUT4 ($P<0.001$). Omentin per se, had no significant effect on either glucose uptake or GLUT4 and showed no additive effect with insulin. In DM, insulin increased glucose uptake, although to a lesser degree than in NG. In DM, omentin (300ng/ml), when added to 25 or 100uU/ml insulin, enhanced glucose uptake compared to 100uU/ml insulin ($P<0.05$ and $P<0.01$, respectively). This enhancement was supported by increments of GLUT4 (10.6±2.6% for 300ng/ml omentin +25uU/ml insulin, 15.9±4.4% for 300ng/ml omentin +100uU/ml insulin vs 7.9±2.8% for 100uU/ml insulin, $P<0.05$).

Conclusions: Omentin had no additive effect to insulin in monocytes from NG. On the other hand in DM, where insulin's action was defective, omentin enhanced insulin-stimulated glucose uptake in monocytes, partially compensating insulin's failure to produce a maximal effect.

NBDG uptake (%increment from blank)	w/o insulin, omentin	25uU/ml insulin	100 uU/ml insulin	100ng/ml omentin	100ng/ml omentin+ 25uU/ml insulin	100ng/ml omentin+ 100uU/ml insulin	300ng/ml omentin	300ng/ml mentin+ 25uU/ml insulin	300ng/ml omentin+ 100uU/ml insulin
Diabetics (DM)	28.97±3.1	35.84±2.1^{+}	37.52±2.4^{++}	34.67±1.42^{+}	38.22±3.41^{++}	44.48±2.92^{+++}	33.02±2.92	43.23±1.91$^{*,++}$	48.6±3.05$^{**,+++}$
Normoglyceamic (NG)	32.91±2.9	51.3±5.1$^{++,a}$	62.96±6.5$^{+++,aa}$	38±5.8	52±6.3$^{++,a}$	62±5.36$^{+++,aa}$	44.45±8.27	55.47±6.44$^{++,a}$	65±5.96$^{+++,a}$

*p<0.05, **p<0.01 vs 100 uU/ml Insulin
$^{+}$p<0.05, $^{++}$p<0.01,$^{+++}$p<0.001 vs 0 uU/ml Insulin
^{a} p<0.05, ^{aa}p<0.01 vs respective value of DM

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Acute effects of estrogen receptor agonists on vascular reactivity of diabetic ovariectomised rats

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Background and aims: Estrogen protects against cardiovascular disease in premenopausal women. These cardioprotective effects are absent in diabetic and postmenopausal women. But the relative role of estrogen receptors (ER-alpha; ER-beta) is not clear in estrogen-induced vasorelaxation of these situations. The aim of the study was to determine how individual estrogen receptor isoforms modulate vascular reactivity in diabetic ovariectomized rats.
Materials and methods: 4 groups of Wistar rats have been used in this study. Control group (C;n=8), ovariectomized group (OVX; n=13), diabetic group (DIA;n=5), diabetic ovariectomized group (DIA+OVX; n=7). Bilateral ovariectomy had been performed to anesthetized (ketamine+xylasine) animals. Diabetes induced by a single i.v.injection of streptozotocin (45 mg/kg) a week after the operation. After 8 weeks, thorasic aortae were removed and mounted in organ baths for measuring isometric relaxations. Experiments were carried out on rings precontracted with phenylephrine (PE) to 60% of maximal contraction. Cumulative concentration-response curves for 17-beta estradiol (E2; nonselective agonist), 4,4',4"-(4-propyl-[1H]-pyrazole-1,5-triyl) tiphenol (PPT;ER-alpha selective agonist) and diarylpropionitrile (DPN;ER-beta selective agonist) were obtained with (10^{-13} - 10^{-6} M) concentration range.
Results: An increase in body weight was observed in OVX group (p<0.001 vs C group) but not in the diabetic groups. Blood glucose levels were increased in the DIA and OVX+DIA group compared with the C group (p<0.001). Plasma estradiol levels and the ratio of uterine weight/body weight were reduced in OVX,DIA and OVX+DIA group compared C group respectively (p<0.01;p<0.001) The vasorelaxation responses to estrogenic agonists E2 and PPT were reduced in OVX, DIA and OVX+DIA however these responses to DPN were reduced both OVX groups (p<0.001).The ratios % of the maximum relaxations with E2, PPT and DPN of PE-precontracted aortic rings were shown in Table(***p<0.001 vs C).
Conclusion: These findings suggest that ER-alpha has still play a vital role in diabetes or ovariectomized vessels. This study also support that the estrogenic vasodilatation is abolished under diabetic ovariectomized condition which may be contribute to diabetic postmenopausal vascular dysfunction.

The % of the maximum relaxations with E2, PPT and DPN of PE-precontracted aortic rings

Groups	E2	PPT	DPN
C	24.0 ± 0.1	29.6 ± 1.0	17.0 ± 0.4
OVX	16.6 ± 0.4***	18.0 ± 6.3***	6.9 ± 1.0***
DIA	16.1 ± 1.1***	19.6 ± 1.2***	12.0 ± 1.4
OVX+DIA	8.5 ± 0.5***	9.0 ± 0.6***	8.0 ± 1.3***

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Differential role of testosterone and estradiol on glucose and lipid metabolism in human skeletal muscle cells

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Background and aims: Testosterone and estradiol ratio differs between sexes, where changes in sex hormones homeostasis may cause different pathological response. We have previously shown that human skeletal muscle cell culture obtained from elderly female and male healthy subjects showed no intrinsic sex differences on glucose and lipid metabolism. Aim of the study is to determine whether testosterone or estradiol treatment have different role on lipid and glucose metabolism in human skeletal muscle cell culture.
Materials and methods: Myotubes obtained from women and men donors were treated with 10 nM testosterone or estradiol for 4 days. Insulin-stimulated glycogen synthesis and palmitate oxidation were assessed and samples were collected for immunoblot analysis to assess AMPK, AKT, ERK1/2 and p38 MAPK phosphorylation and total protein. mRNA levels of different metabolic genes were also determined using real-time PCR analysis.
Results: Testosterone enhanced glucose incorporation to glycogen and insulin-stimulated Akt phosphorylation specifically in myotubes from women, but not from men, donors, indicating sex specific role of testosterone on glycogen synthesis in skeletal muscle. Testosterone enhanced both AMPK phosphorylation and lipid oxidation in myotubes obtained from both sexes. mRNA expression showed a differential response to either sex hormone treatment with no sex differences. Testosterone increased the glycogen synthase 1 (GYS1), while estradiol altered the mRNA expression of stearoyl-CoA desaturase (SCD) and Pyruvate dehydrogenase kinase 4 (PDK4).
Conclusion: Only testosterone treatment showed an effect on lipid metabolism while, both sex hormones changed the mRNA expression of some genes involved in lipid oxidation. In this study, we are able to suggest an important role of testosterone on glucose and lipid metabolism in human skeletal muscle cells, with no clear effect of estradiol on metabolism and there is a sexual difference on cellular metabolism with sex hormones treatment.

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Circulating pigment epithelium-derived factor levels are associated with insulin resistance and decrease after weight loss

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Background and aims: Pigment Epithelium-Derived factor (PEDF) is a 50-kDa protein with anti-inflammatory activity. We aimed to study PEDF in vivo in association with insulin resistance and in vitro in human adipocytes.
Materials and methods: Circulating PEDF (ELISA) and metabolic profile were assessed in 125 Caucasian men. PEDF levels were also assessed in an independent cohort of subjects (n=33) to study the effects of changing insulin action. PEDF gene expression and PEDF secretion were also measured during differentiation of human preadipocytes.
Results: In all subjects, PEDF were positively associated with BMI (r=0.326, p<0.0001), waist-to-hip ratio (r=0.380, p<0.0001), glycated hemoglobin and fasting triglycerides; and negatively with insulin sensitivity (r=-0.320, p<0.0001). Circulating PEDF levels was significantly increased in subjects

with altered glucose tolerance and type 2 diabetes. Of the inflammatory markers measured, circulating PEDF levels were positively associated with serum sTNFR1 and IL-10 in obese subjects. Interestingly, weight loss led to significantly decreased of PEDF concentration from 34.8±19.3 μg/ml to 22.5±14.2 μg/ml (p<0.0001). Multiple linear regression analyses revealed that insulin sensitivity contributed independently to explain 14% of the variance in circulating PEDF levels, after controlling for the effects of BMI, age, and log fasting triglycerides. Differences in circulating total PEDF observed after weight loss were strongly related to changes in obesity and insulin resistance measures. *PEDF* gene expression and PEDF secretion increased during differentiation of human pre-adipocytes.
Conclusion: Circulating PEDF is strongly associated with insulin sensitivity. The findings show, for the first time in humans, that circulating PEDF concentrations decrease significantly after weight loss in association with improvement of insulin action. PEDF seems to be involved in human adipocyte biology.
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Osteocalcin and its effect on metabolic control in type 1 diabetes
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Background and aims: Osteocalcin (OC), produced by osteoblasts, is considered a predictor of fractures and its elevated levels are associated with both high bone formation and turnover. Recent animal studies have shown that OC acts also as a hormone regulating glucose metabolism and fat mass. Few studies have explored this relationship in humans showing an inverse correlation with fasting plasma glucose, HbA1c and fat mass in type 2 diabetic patients. However, no data are available in type 1 diabetes (T1D). Aim of this study was to investigate the relationship between OC, HbA1c and fasting serum C-peptide in patients with recent onset T1D. Moreover, we tested in T1D patients the effect of 1 year calcitriol administration on OC, residual β-cell function and metabolic control (HbA1c and insulin requirement).
Material and methods: 34 subjects (mean age 22.2±1.7 SE) with recent-onset T1D and baseline C-peptide > 0.25 nM were enrolled in this study. Intensive insulin therapy was implemented with three daily injections of regular insulin + bedtime insulin in these patients. Osteocalcin was analyzed at the time of diagnosis and one year after treatment with either 0.25 μg daily calcitriol or placebo. OC was evaluated by ECLIA method (modular E170, Roche Diagnostics, Mannheim, Germany) in relation to C-peptide.
Results: At *T1D diagnosis*, OC levels were significantly lower in female than in male patients (17.8±3.1 ng/mL vs 43.9±6.9 ng/mL; p<0.01) while no other metabolic parameters as HBA1c, C-peptide differed between gender. Analyzing the whole group of patients, OC levels were positively associated with insulin requirement (r=0.48, P<0.01), while no significant correlation were found in relation to HbA1c and C-peptide. *At 1 year follow-up,* OC levels were increased (11%) in the placebo group while dropped by 25% in the calcitriol group, but their levels were not significantly different compared to diagnosis due to high variability. No significant differences were observed between calcitriol and placebo groups for OC (37.2±11.8 ng/mL vs 25.8±7.4 ng/mL; p=NS), C-peptide(1.05±0.25 nM vs 25.8±7.4 nM; p=NS), HbA1c (6.8±1.13% vs 7.14±1.1%; p=NS) and insulin requirement (0.48±0.2 vs 0.58±0.21; p=NS).
Conclusion: In contrast to what expected, OC was not associated with residual pancreatic β-cell function or metabolic control in recently diagnosed T1D patients. Calcitriol had no effect on residual β function, insulin dose or the overall metabolic control. Further studies with a larger group of patients are needed to confirm these data.

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The relationship between vitamin D status and markers of oxidation and inflammation in subjcets with the metabolic syndrome
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Background and aims: Low levels of vitamin D may increase the risk of developing type 2 diabetes, and inflammation and oxidative stress may participate in the pathogenesis of diabetes. We have evaluated the relationship between serum 25-hydroxyvitamin D_3 ($25(OH)D_3$) and markers of inflammation and oxidative stress in subjects with the metabolic syndrome (MetS).
Material and methods: $25(OH)D_3$ was measured with HPLC-MS in subjects with the MetS in the LIPGENE dietary intervention study. CRP was measured by ELISA and urinary 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) and 15-keto-dihydro-prostaglandin $F_{2\alpha}$ (15-keto $PGF_{2\alpha}$) were determined by RIA and adjusted for urinary creatinine. Subjects (n = 446, 45% males) were from eight European centres. Mean (SD) age was 54.7 (9.0) years and BMI 32.3 (4.1) kg/m^2.
Results: The mean (SD) concentration of $25(OH)D_3$ was 57.1 (26.0) nmol/L. Subjects were grouped according to their vitamin D status; severely deficient (<25 nmol/L, n=20), deficient (25-49.9 nmol/L, n=189), insufficient (50-74.9 nmol/L, n=146) and sufficient (> 75nmol/L, n=91). Markers of oxidative stress (8-iso-$PGF_{2\alpha}$ p=0.022) and inflammation (15-keto $PGF_{2\alpha}$, p=0.022 and CRP, p=0.05) differed significantly across categories of $25(OH)D_3$. Plasma concentrations of $25(OH)D_3$ were significantly negatively associated with BMI (r=-0.23, p<0.001), CRP (r=-0.11, p=0.02) and 15-keto $PGF_{2\alpha}$ (r=-0.10, p=0.039), but not with 8-iso-$PGF_{2\alpha}$ (r=-0.08, p=0.09).
Conclusion: In a large sample of subjects with the metabolic syndrome low plasma concentrations of $25(OH)D_3$ were associated with enhanced markers of inflammation and oxidative stress.
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PS 65 Herbology in diabetology

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Arab herbal medicine-based combination of four anti-diabetes plants stabilises a physiological blood glucose level

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Background and aims: Medicinal plant-based drug discovery provides important leads against various pharmacological targets including diabetes, which represents a worldwide predominant public health concern. Several drugs are used to treat this disease. Based on our traditional knowledge, *Juglans regia* L., *Olea europea* L., *Urtica dioica* L., and *Atriplex halimus.* L. exhibit favorable effects on blood glucose levels. This study was aimed at investigating safety and efficacy of a fixed mixture of these plants.
Materials and methods: In the present study we assessed safety and anti-diabetic effects of the combination of the four plants leave extracts using *in vivo* (human type2 diabetic volunteers and Streptozotocin-induced diabetic rats) and *in vitro* test systems (human fibroblasts and skeletal muscle cells treated with increasing concentrations of Plant mixture).
Results: No sign of toxic effects were seen in cultured human fibroblasts and skeletal muscle cells treated with increasing concentrations of Plant mixture. Anti-diabetic effects were evidenced by inhibition of glucose intestinal absorption (~49%) in a rat gut-segment. Furthermore, treatment with these plant combined extracts of Streptozotocin-induced diabetic rats for 2-3 weeks, showed a significant reduction in glucose levels [above 400±50 mg/dl to 210±22 mg/dl (P<0.001)] and significantly improved sugar uptake during the glucose tolerance test, compared with positive control. In addition, glucose levels were tested in sixteen human volunteers, with the recent onset of type 2 diabetes mellitus, who received the plants mixture tablets 1X3 daily for a period of 4 weeks. Within the first week of the tablets consumption, baseline glucose levels were significantly reduced from 290±40 to 210±20 mg/dl. At baseline, a subgroup of eleven of these subjects had glucose levels below 300mg/dl and the other subgroup had levels ≥300 mg/dl. Clinically acceptable glucose levels were achieved during the 2-3 weeks of therapy in the former subgroup and during the 4th week of therapy in the latter subgroup. No side effect was reported.
Conclusion: Results demonstrate safety, tolerability and efficacy of herbal combinations of four plants that seem to act differently but synergistically to regulate glucose-homeostasis.

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Effects of Mongolian traditional medicinal plant extract of Gentianaceae on *in vivo* insulin action in streptozotocin-induced diabetic rats

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Background and aims: Lomatogonuim rotatum (L) Fries ex Fern and Gentiana acuta Michaux of the family Gentianaceae have been documented as traditional plant treatments for liver diseases.We firstly performed a preliminary investigated on the acute effect of Mongolian medicinal plant extracts Lomatogonuim rotatum and Gentiana acuta , Gentianaceae on the blood glucose tolerance in rats, evaluated by an oral glucose tolerance test (OGTT). Then, investigated the effects of the same extracts on in vivo insulin action in streptozotocin (50mg.kg-1BW,i.v.)-induced diabetic rats by means of the euglycemic clamp.
Materials and methods: Plant materials were provided by the Institute of Chemistry and Chemical-technology of MAS (Mongolia). Air-dried plant materials were extracted with 10 volumes of hot water (80C), filtered, lyophilized, and stored at 40C. Male Wistar rats aged 8 weeks (220-240g bw).Oral glucose tolerance test (OGTT). Time-course blood glucose concentrations were determined 30, 60, and 120 min after glucose load. Whole blood and plasma assays. Blood glucose concentration was determined using an automated analyzer model 2300 STAT Plus. Plasma insulin concentration was measured by the Special Reference Laboratories Inc. using a chemiluminescence enzyme immunoassay method. Statistical analysis: Data were analyzed by one-way analysis of variance. When a significant difference was found ($P < 0.05$), values were further compared with the Student's t test. The Stat View was used for statistical analyses. Data are expressed as means ± SE.
Results: 1.Effect of plant extract on blood glucose concentrations. Blood glucose concentrations and net incremental area under the curve (net AUC) for control and plant extracts before and after oral load of glucose are summarized. Compared to the control group, plant extract groups (30min) had significantly lower peak blood glucose concentrations during the OGTT with Lomatogonium rotatum and Gentiana acuta, Gentianaceae. 2. Effect of 7 days administration of plant extracts on MCR Rats divided into control and 7-days oral administration groups. At low-dose insulin infusion, the decreased metabolic clearance rates of glucose (MCR) in diabetic rats were improved by a 7 days administration of plant extracts (500mg.kg.BW, p.o.; 7 days effect: non-diabetic control: 15.2± 0.8 ml kg-1min-1, diabetic control: 7.6± 0.7ml kg-1min-1. versus plant extract 1: 14.1 ± 1.4 ml kg-1min-1, and versus plant extract 2: 13.2 ± 1.2 ml kg-1min-1, P<0.001, respectively).
Conclusion: These results suggest that a 7 days administration of the Mongolian plant extracts Gentianaceae can improve glucose utilization and insulin resistance in diabetic rats.
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Chronic caffeine intake reverses age induced-insulin resistance in the rat

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Background and aims: Aging is known to be associated with increasing insulin resistance and declining glucose tolerance. Insulin resistance is one of the core metabolic abnormalities in type 2 diabetes and metabolic syndrome. One of the lifestyle changes advised in these diseases is coffee withdrawal, based on several studies describing that caffeine can acutely lower insulin sensitivity. However the benefits of coffee withdrawal have been questioned by several studies that suggest no association between long-term coffee consumption and diabetes which seems to indicate that acute and chronic intake have opposite effects. In the present work we tested the hypothesis that chronic caffeine intake reverses age induced-insulin resistance in the rat and investigated if the mechanism by which caffeine restores insulin sensitivity is due to a decrease in visceral fat or oxidative stress.
Materials and methods: Six groups of rats were used: 3 months old (control), 3 months caffeine-treated, 12 months old, 12 months caffeine-treated, 24 months old and 24 months caffeine-treated. Caffeine was administered in drinking water (1g/l) during 15 days. Insulin sensitivity was assessed by means of an insulin tolerance test. Blood pressure, weight, visceral and total fat, basal glycemia, insulinemia and plasma total antioxidant capacity were also measured.
Results: Insulin sensitivity diminished in 12 and 24 months rats as the constant of the insulin tolerance test (K_{ITT}) decreased significantly to 2.77±0.17 and 2.47±0.18 compared to the control value 4.69±0.42 (3 months rats). Chronic caffeine intake restored insulin sensitivity to control values both in 12 and 24 months rats. Basal glycemia was 100.53±4.32 mg/dL, 96.87±3.43 mg/dL and 96.63±3.10 mg/dL in 3, 12 and 24 months rats. Caffeine did not modify basal glycemia in any of the groups tested. Both 12 and 24 months rats were hyperinsulinemic, as insulin levels increased significantly by 169 and 160%, respectively from a control value of 2.05±0.4 µg/L. Chronic caffeine intake significantly decreased plasma insulin levels ($p<0.5$) both in 12 and 24 months rats, although without restoring plasma insulin to control values. Visceral and total fat were significantly increased both in 12 and 24 months rats when compared with 3 month rats, however no correlations were found between visceral fat and the K_{ITT} in both 12 and 24 months rats ($p=0.06$ and $r=0.88$; $p = 0.77$ and $r= 0.13$, respectively). Caffeine intake significantly decreased visceral and total fat in 12 but not in 24 months rats. Also, no correlations were found between visceral fat and the K_{ITT} in 12 and 24 months old caffeine treated rats ($p=-0.35$ and $r=0.35$; $p = 0.57$ and $r= 0.24$, respectively). Additionally, chronic caffeine intake did not significantly modify the weight of the animals within the groups. Total antioxidant capacity (TAC) was decreased in 12 and 24 months rats. Caffeine intake did not modify significantly TAC in any of the groups tested.
Conclusion: Chronic caffeine intake reverses age induced-insulin resistance in rats, an effect that were independent of weight loss, visceral fat and oxidative stress.
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Shikonin improves blood glucose levels in diabetic GK rats and increases glucose uptake in adipocyte and muscle cells, independent of its effect on NADPH oxidase

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Background and aims: Shikonin is a naphtoquinone derivative from the Chinese plant *Lithospermum erythrorhizon*. It has been reported to inhibit the formation of the NADPH oxidase, an enzyme complex that catalyses the formation of highly reactive superoxide anions. Shikonin has been shown to stimulate glucose-uptake, and potentiate insulin-induced glucose-uptake in 3T3-L1 adipocytes. The diabetic state is associated with excess superoxide production that may contribute to failure of insulin to stimulate glucose uptake in fat and muscle. Hence, our aim was to investigate the effect of shikonin, administered intraperitoneally (i.p.), on blood glucose levels and insulin sensitivity in the spontaneously diabetic Goto-Kakizaki (GK) rats. Having found that shikonin improved glucose homeostasis in GK rats, we further studied its mechanism of action in vitro.
Materials and methods: Shikonin was given i.p. to GK rats (n=6) once daily (10 mg/kg) for 4 days and compared with placebo (vehicle DMSO/oil (9:1)) injected i.p. during 4 days in the same animals. Plasma glucose (PG) levels were measured daily before and 6h after i.p. injections. At the 4th day, an insulin sensitivity test was performed, where glucose responses were measured after s.c. injection of 0.5 U/kg insulin. L6 muscle cells and 3T3-L1 cells were used as model systems to study how shikonin regulates glucose uptake. Glucose uptake was measured using the 2-deoxy-[^{3}H]-D-glucose method. AMPK and Akt phosphorylation were determined by Western blot. Oxygen consumption was monitored with a Clark-type oxygen electrode.
Results: Shikonin significantly lowered morning PG on 2^{nd,} 3rd and 4th days compared to day 1 ($p < 0.01$ for all days compared to the first day); the total area under the glucose curve was lower in shikonin treated rats vs control rats (p= 0.014). In the insulin sensitivity test, PG levels were more reduced in the shikonin treated rats; 30-240 min after injection of insulin, the areas under the PG curves (AUCs) being 39.3±105.9 and 536.4 ±144.0 mM/210 min, respectively ($p<0.02$). Shikonin increased basal and insulin-stimulated glucose uptake in L6 cells and 3T3-L1, which does not express all subunits of NADPH oxidase. Shikonin did not mimic the effect of AMPK activator AICAR on AMPK-phosphorylation, i.e. AMPK was not phosphorylated by shikonin. Furthermore, shikonin did not induce any change in AMP-to-ATP ratio and Akt phosphorylation in L6 cell lines, nor did it increase oxygen consumption in skeletal muscle mitochondria. However the cell-permeable calcium chelator, BAPTA-AM (5 μM), blocked shikonin-stimulated glucose uptake in L6 cell lines.
Conclusion: We conclude that shikonin treatment in GK rats decreases PG levels and improve insulin sensitivity. Since shikonin increased glucose uptake in cell lines devoid of NADPH oxidase, this enzyme cannot be involved in shikonin-mediated effects on glucose metabolism. Our present findings suggest that shikonin exerts its effect on glucose uptake by a calcium related pathway.

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Effect of resveratrol on insulin sensitivity, oxidative stress and Akt pathway in humans

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Background and aims: To examine whether the red wine polyphenol, resveratrol, improves insulin sensitivity in type 2 diabetic patients, and to gain insight into the mechanism of its action.

Materials and methods: After an initial general examination (including blood chemistry), 19 patients enrolled in the 4-week long study were randomly assigned into two groups: a resveratrol group receiving oral 2 x 5 mg resveratrol and a respective control group receiving placebo. Before, after two weeks and at the end of the trial insulin resistance/sensitivity, creatinine-normalized ortho-tyrosine level in urine samples (as a measure of oxidative stress), incretin levels and pAkt/Akt ratio in platelets were assessed and statistically analyzed.
Results: After 4 weeks, resveratrol significantly decreased insulin resistance (HOMAIR) and urinary ortho-tyrosine excretion, while it increased pAkt/Akt levels in platelets. On the other hand, it had no effect on parameters characterizing beta cell function.
Conclusion: This study shows the first time that resveratrol improves insulin sensitivity in humans, which might be due to a resveratrol-induced decrease in oxidative stress that leads to a more efficient insulin signaling via the Akt pathway.

PS 66 Liver, hepatic steatosis and metabolism

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An analysis of liver enzymes in type 1 and type 2 diabetes and their associations with glycaemic status independent of total adiposity

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Background and aims: Robust evidence indicates hepatic fat and related liver enzymes, alanine transaminase [ALT] and gamma glutamyl transferase [GGT], predict type 2 diabetes. However, the association of these enzymes with glycaemic control in people with diabetes is less well studied. We therefore investigated whether there is an association between liver enzymes and glycaemic control independently of age, sex, adiposity and smoking status in type 1 and type 2 diabetes
Materials and methods: We used population-based diabetes register data for 2008 linked to laboratory data for 3862 people with type 1 and 25075 people with type 2 diabetes in south-east Scotland. Regression analyses were used to examine associations between ALT or GGT and HbA1c in people with type 1 and type 2 diabetes controlling for age, sex, current smoking status and body mass index (BMI); after excluding people with ALT and GGT values more than twice the upper limit of normal (>2ULN) and people with HbA1c>10% (associations were not linear beyond these values). Data on alcohol intake were not available.
Results: Data were available for ALT and GGT on 27258 (94%) and 25840 (89%) people respectively. There were 1447 people (5.3%) whose ALT was >2ULN (>100U/l), 4662 people (18.0%) whose GGT was >2ULN (>110U/l) and 1972 (6.9%) people whose HbA1c was >10% leaving data available for analysis for 2416 people with type 1 and 19058 people with type 2 diabetes. ALT was 9.9 U/l higher ($p<0.0001$) and GGT was 10.5 U/l higher ($p<0.0001$) in people with type 2 compared to type 1 diabetes after adjustment as described above. BMI≥30kg/m2 was associated with higher levels of ALT (2.63 U/l, $p<0.001$) and GGT (4.65U/l, $p<0.001$) when compared to BMI<30kg/m2. Current smoking compared to non-current smoking status was associated with lower ALT levels (by 1.84 U/l, $p<0.001$) and higher GGT levels (by 1.94 U/l, $p<0.001$). In fully adjusted analyses, each 1% increase in HbA1c was associated with: a) no significant change in ALT in type 1 diabetes but a 0.90 U/l increase in type 2 diabetes ($p<0.0001$); and b) a 0.73 U/l increase in GGT ($p=0.04$) in type 1 diabetes, and a 1.23U/l ($p<0.0001$) increase in type 2. There was a significant ($p<0.001$) interaction between obesity (BMI≥30kg/m2) and GGT with HbA1c as the outcome for people with type 2 diabetes in adjusted analyses
Conclusion: The relationship between glycaemic control and either ALT or GGT differs by diabetes type. The data provide strong, albeit indirect, evidence for an influence of hepatic fat accumulation (independent of total adiposity) on glycaemic control in type 2 diabetes. Further work is required to establish the role of alcohol consumption and diabetes drugs on such patterns and to investigate whether improved glycaemic control results in reduction of liver enzymes and / or vice versa.

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Serum interleukin 1 receptor antagonist level is independently associated with nonalcoholic steatohepatitis in humans

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Background and aims: Mechanisms leading to non-alcoholic steatohepatitis (NASH) have remained unclear, and noninvasive diagnosis of NASH is challenging. In this study we aimed to identify novel serum markers for NASH.
Materials and methods: In a cross-sectional population-based cohort of 6447 men (58±7 years, BMI 27.0±3.9 kg/m2) the association of serum ALT levels with glucose tolerance, Matsuda insulin sensitivity index (ISI), serum lipids and lipoproteins, and serum levels of adiponectin and cytokines were investigated. Liver biopsies from 60 morbidly obese individuals (44.2±8.3 years, BMI 45.5±6.1 kg/m2) were used for histological assessment. Gene expression of IL1RN in liver, subcutaneous fat and visceral fat was investigated.
Results: The strongest determinants of ALT levels were Matsuda ISI and serum IL-1RA levels in the population study. IL-1RA levels associated significantly with ALT levels even after adjusting for BMI, alcohol consumption and insulin sensitivity (general linear model, p=2x10-21). In morbidly obese subjects serum levels of IL-1RA also associated with the degree of lobular inflammation in liver histology ($p=0.034$). Furthermore, serum IL-1RA levels decreased after obesity surgery ($r=0.443$, $p=0.024$) and this decrease correlated with the change in histologically assessed lobular inflammation ($r=0.662$, $p=0.027$). Finally, expression of IL1RN in liver and visceral fat correlated positively with serum IL-1RA levels and liver steatosis ($r=0.352$ and 0.462, respectively, $p<0.05$).
Conclusion: IL-1RA serum levels correlate with serum ALT independent of obesity, alcohol consumption and insulin resistance, most likely reflecting an association between serum IL-1RA and NASH.
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Increased intramyocellular lipid but normal intrahepatocellular lipid content characterises polycystic ovary syndrome compared with age- and BMI-matched healthy controls

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Background and aims: It has been suggested that there is a high prevalence of non-alcoholic fatty liver disease (NAFLD) in women with Polycystic Ovary Syndrome (PCOS) as both are associated with obesity and insulin resistance (IR). Furthermore, the mechanism for the peripheral insulin resistance observed in PCOS remains unclear. The aim of this study was to determine whether PCOS women have higher liver fat (intrahepatocellular lipid, IHCL) or skeletal muscle fat (intramyocellular lipid, IMCL), compared with age- and body mass index-matched controls, and to determine whether higher tissue fat content may explain IR.
Materials and methods: 20 PCOS women and 9 healthy controls were recruited. Fasting glucose, lipids, AST and ALT were measured and all subjects underwent whole body magnetic resonance imaging with proton magnetic resonance spectroscopy to determine IHCL and IMCL (soleus, SOL and tibialis anterior, TA) levels.
Results: PCOS women and healthy controls were similar with respect to BMI and age (32 ± 8 vs. 28 ± 5 kg/m$^{2;}$ 26 ± 4y vs. 28 ± 7y). PCOS women had higher fasting triglycerides but similar liver transaminases and similar IHCL (8 ± 13% vs. 3 ± 4%; $p=0.09$). IHCL was significantly correlated with BMI and waist:hip ratio (WHR) in PCOS and control women. At any given BMI or WHR, PCOS women and control women had similar IHCL. IHCL was related to serum triglycerides. There was also a close correlation between IMCL and BMI. IMCL content was greater in the TA muscle in PCOS vs. control (77 ± 56% vs. 41 ± 30%; $p<0.05$) but not in soleus (54 ± 38% vs. 34±24; $p=0.1$).
Conclusion: Despite the reported high prevalence of NAFLD in PCOS we found that IHCL was elevated similarly in proportion to the BMI in both PCOS and healthy controls unlike IMCL which was higher in women with PCOS and may potentially explain peripheral insulin resistance.

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Proteasome dysfunction contributes to endoplasmic reticulum stress and insulin resistance in type 2 diabetic liver

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Background and aims: Insulin resistance is a key feature of people with type 2 diabetes (T2D). Growing evidence has suggested that accumulation

of endoplasmic reticulum (ER) stress in liver is a major contributor to insulin resistance; however, the molecular mechanisms linking diabetes and ER stress are not fully understood. We have previously reported that the hepatic expression of genes involved in proteasomal degradation pathway are coordinately up-regulated in people with obesity and T2D (Obesity 2008). Specifically, the expression levels of proteasome activator (PA) 28-α subunit gene was significantly higher in people with obesity than in non-obese individuals. The proteasome is an important multicatalytic enzyme complex that degrades misfolded and oxidized proteins, signal transduction factors, and antigenic peptides for presentation. Indeed, NEFA or oxidative stress have been reported to induce proteasome dysregulation in hepatocyte. The aim of this study is to clarify the role of proteasome function for insulin resistance in obesity and T2D using PA 28α, β, and γ triple knockout (KO) mice.

Materials and methods: We assessed the metabolic phenotype of PA28αβγ KO mice. After sacrifice, blood sample, liver and femoris muscle were collected and proteasome activity was measured by the chymotrypsin-like protease activity. Livers were observed by electron microscope. Gene and protein expression of markers associated with ER stress was analyzed by realtime-PCR and Western blot.

Results: 1) Proteasome activity was inhibited in livers of both genetically diabetic db/db mice and C57BL/6 mice fed a high-fat diet (HFD) ($p<0.05$; $p=0.083$, $n=4$), whereas gene expression levels involved in proteasomal degradation pathway were up-regulated in livers of mice fed a HFD. 2) Proteasome activity in livers of PA28αβγ KO mice was inhibited by 35% compared with control mice ($p=0.05$, $n=3$). 3) PA28αβγ KO mice showed glucose intorelance in glucose loading test. 4) Insulin-stimulated phosphorylation of Akt was impaired in livers of PA28αβγ KO mice, but not in skeletal muscle. 5) Western blot analysis displayed an accumulation of polyubiquited proteins in the liver of PA28αβγ KO mice. 6) Electron microscopic examination detects a massive expansion of endoplasmic reticulum and double-membrane and multilamellar structures of large autophagosomes in hepatocytes from PA28αβγ KO mice. 7) CHOP and spliced XBP-1 mRNA level were increased in livers from PA28αβγ KO mice ($p<0.05$; $p=0.077$, $n=3$-5). 8) GRP78, pPERK, peIF2α, pIRE-1, CHOP, and pJNK protein levels were up-regulated in livers obtained from PA28αβγ KO mice ($p<0.05$, $n=5$).

Conclusion: Proteasome dysfunction induces ER stress and subsequent insulin resistance in the liver with obesity and T2D. Our study demonstrates a previously unrecognized role of ubiquitin-proteasome pathway for the development of insulin resistance in the liver and suggests that this pathway is a novel target for the treatment of T2D.

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Berberine reduces hepatic fat content in SD rats with a high-fat diet by increasing hepatic MTTP function

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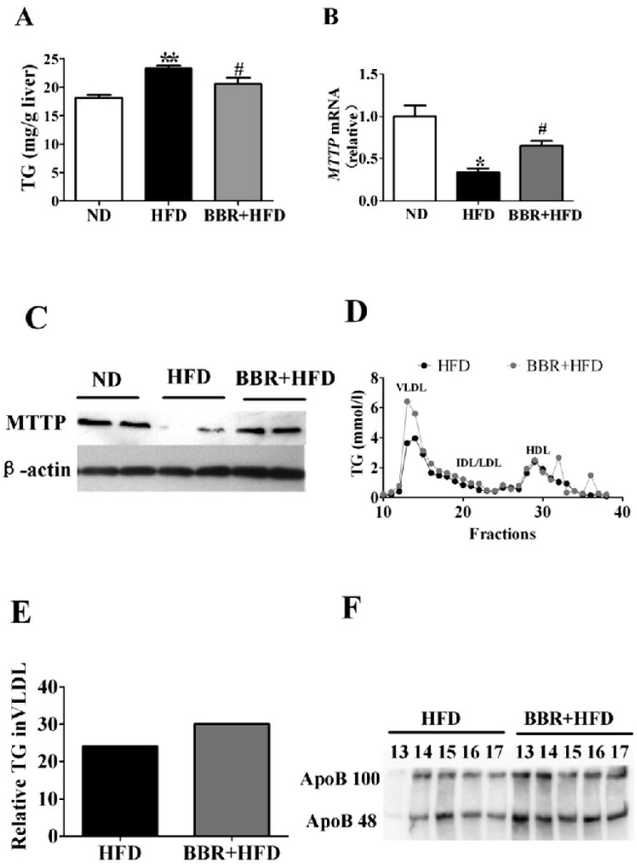
Background and aims: Nonalcoholic fatty liver disease (NAFLD) is closely associated with obesity, insulin resistance, and type 2 diabetes. Hepatic fat content plays a key role in these disorders, so reducing hepatic fat accumulation can be an effective strategy to prevent type 2 diabetes. In our previous study, we found that berberine (BBR) can reduce hepatic triglyceride content and decline the DNA methylation level of hepatic microsomal triglyceride transfer protein (MTTP) promoter to upregulate MTTP expression. MTTP is necessary for the assembly and secretion of apoB-containing lipoproteins (e.g.VLDL, LDL), whereby lipids are normally exported from the liver. Hypermethylation of MTTP promoter reversed by BBR treatment, but it is unclear whether BBR improve hepatic MTTP function. So we further explored the mechanism of BBR reducing liver fat content by regulating MTTP.

Materials and methods: After Sprague- Dawley (SD) rats (n=16) were fed with 8 weeks of high-fat diet (HFD, 51% energy from fat, 4.64 kcal/g), NAFLD model was successfully established. Then rats with NAFLD were randomly divided into two groups, one of which were treated with BBR orally at 200mg/(kg d) (n=8, BBR+HFD group) and another group fed with vehicle (0.5% methylcellulose) as HFD control (n=8, HFD group), for sixteen weeks. Meanwhile, SD rats with normal diet (12.5% energy from fat, 3.2kcal/g) received the vehicle as normal control (n=8, ND group). At the end of the experiment, all rats were sacrificed and samples of liver tissue were taken for quantitative real-time PCR analysis and hepatic fat content measurement after overnight fasting. Total blood samples were also collected for serum lipoprotein profiles.

Results: Liver triglyceride content is significantly lower in BBR treatment group than in HFD group ($p<0.05$, Fig A), although it was higher than in the normal control. The results of quantitative real-time PCR showed that treated with BBR for 16 weeks upregulated hepatic MTTP mRNA level approaching that of normal control ($p<0.05$, Fig B), and MTTP gene expression declined by 70%in livers from rats in the HFD group in contrast to ND group. Accordingly, the protein levels of MTTP were higher in BBR-treated group than in HFD group (Fig C). Serum lipoproteins were separated using fast protein liquid chromatography (FPLC) (Fig D). The contents of apoB100 and apoB48, in the fractions were visualized by Western blotting. These results showed that TG-rich VLDL particles were significantly higher in BBR-treated group than HFD group according to area under the curve (AUC) (Fig E) and the levels of apolipoprotein B (apoB) -100 and -48 in isolated VLDL fractions were higher in BBR-treated group than HFD group (Fig F).

Conclusion: BBR can improve fatty liver by upregulating MTTP expression to increase hepatic VLDL-TG secretion in SD rats induced by a high-fat diet.



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cAMP response element binding protein H, CREBH decreases hepatic lipogenesis

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Background and aims: Nonalcoholic fatty liver disease (NAFLD) is closely associated with insulin resistance state, such as obesity and type 2 diabetes. Sterol regulatory element binding protein-1c (SREBP-1c) is one of the major regulators of the expression of genes involved in hepatic triglyceride synthesis. Hepatic expression of SREBP-1c is regulated by insulin-induced activation of liver X receptor (LXR) and specific protein 1 (Sp1). Endoplasmic reticulum-bound transcription factor families are shown to be involved in the control of various metabolic pathways. Here we show a novel function of

ER-bound transcription factor, cAMP response element binding protein H (CREBH), in the regulation of hepatic lipogenesis
Materials and methods: To demonstrate that CREBH expression is influenced by insulin, we determined hepatic CREBH expression during fasting and after the refeeding of control and streptozotocin-induced diabetic rats. We next examined whether CREBH decreased hepatic lipogenesis and SREBP-1c expression in high fat diet fed mice, using tail vein injection of adenovirus encoding the active form CREBH. Finally, we examined the mechanism by which CREBH inhibits *h*epatic SREBP-1c expression.
Results: We found that fasting induced but feeding suppressed CREBH expression. However, feeding did not suppress its expression when endogenous insulin was eliminated by treatment with streptozotocin. Insulin treatment decreased CREBH expression in cultured hepatocytes, suggesting that the refeeding-suppression of CREBH expression is mainly mediated by insulin. Adenovirus-mediated overexpression of CREBH inhibited insulin- and LXR agonist, TO901317-stimulated SREBP-1c mRNA expression in cultured hepatocytes. Moreover, adenovirus-mediated overexpression of CREBH inhibited hepatic steatosis in animal models through an inhibition of SREBP-1c expression. Transient transfection and gel shift assays showed that CREBH inhibited the activities of LXR and Sp1, known mediators of insulin-dependent SREBP-1c expression.
Conclusion: Collectively, these data suggest that CREBH could be a novel negative regulator of hepatic lipogenesis. This raises the possibility that CREBH can be therapeutic target to prevent the development of fatty liver disease in patients with insulin resistance.

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The effect of metformin on liver mitochondria and lipid metabolism in NAFLD

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Background and aims: Metformin is a weak inhibitor of complex I of mitochondrial respiratory chain (MRC) what may negatively influence the cellular energy balance. Our study was designed to determine the effect of long-term high-fat diet administration in combination with metformin treatment on the lipid metabolism in liver, oxidative capacity of liver mitochondria and sensitivity of the liver to the ischemia reperfusion injury.
Material and methods: Male Wistar rats were fed standard (SD) or high-fat diet (HFD, 60% of calories derived from lard) for 10 weeks. Half of the HFD group was administered metformin in food (150 mg/kg b.wt.) for the last 5 weeks of the feeding period. In a separate experiment, animals were subjected to the warm liver ischemia induced by 20 min clamping of portal vein 2 days prior decapitation.
Results: HFD resulted in TAG accumulation in liver (3±0.3 vs 38±4.6 umol/g; p<0.001) and diminished liver glycogen content (219±18 vs 280±13 umol/g p<0.05). The effect of the diet was further potentiated by metformin (TAG: 73±2.5 umol/g, p<0.001; glycogen 169±6 umol/g, p<0.01). The increased activity of liver lysosomal lipase (LAL) in HFD group suggests the enhanced breakdown of endogenous TAG. Concomitantly, an increased expression of diacylglycerolacyltransferase-1 (DGAT-1) was found what indicates the increased FA reesterification. Metformin in combination with HFD further increased both LAL activity and DGAT-1 expression compared with HFD alone. The stimulatory effect of metformin on endogenous TAG degradation is supported by the elevated ketogenesis in HFD+Met group. HFD decreased the oxidative capacity of liver mitochondria on all tested substrates (glutamate + malate p=0.045; glutamate + malate + ADP p= 0.05; palmitoylcarnitine p=0.043; succinate p=0.051). Metformin potentiated the deleterious effect of HFD on mitochondria but only in animals that underwent liver ischemia (HFD+Met vs HFD: glutamate + malate + ADP p=0.042; palmitylcarnitoin p=0.025). We proved 40% decrease of in vitro activity of NADH:cytochrome c oxidoreductase in HFD+Met vs HFD alone. Liver ischemia resulted in the increased formation of lipoperoxidation products (HFD > SD). Metformin had no additional effect. In animals subjected to the liver ischemia, the activity of antioxidative enzymes (SOD, GSH-Px, catalase) were significantly lower in HFD vs SD group. Metformin treatment of HFD animals resulted in 70-80% increase in the activity of all tested enzymes.
Conclusion: The administration of metformin in combination with HFD worsened hepatic steatosis but it was not associated with the impairment of TAG degradation. On the contrary, the activity of lypolitic enzyme (LAL) was elevated. Metformin also potentiated HFD-induced impairment of mitochondria what deteriorated their ability to utilize released FA by mitochondrial oxidation and re-directed them into ketogenesis. TAG accumulation in liver significantly worsened the oxidative stress in liver ischemia/reperfusion. Metformin stimulated the activity of antioxidant enzymes but this was NOT accompanied by concomitant decrease in lipoperoxide formation. This fact could be interpreted as the adaptive response of the cell to the increased reactive oxygen species formation due to the impairment of MRC.
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Adiponutrin, a lipid droplet surface enzyme - evidence for regulation by ChREBP, SREBP1c and FXR in human hepatocytes

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Background and aims: Adiponutrin, encoded by the PNPLA3 gene, belongs to the family of patatin-like domain containing enzymes. A non-synonymous sequence variation, encoding an isoleucine to methionine substitution at amino acid 148 (rs738409; I148M), has been consistently associated with a markedly increased hepatic fat content in humans with non-alcoholic fatty liver disease. Adiponutrin is reported to show in vitro triglyceride lipase and acyltransferase activities, but its physiologic function and regulation are poorly known. We aim to eludicate (i) transcriptional regulation of PNPLA3, (ii) its subcellular localization in human hepatocytes, and (iii) expression pattern of adiponutrin in human tissues.
Materials and methods: PNPLA3 regulation was studied in a new cell line, immortalized human hepatocytes (IHH). The cells were treated for 24 h with agonists/antagonists of LXR, FXR, PXR, SREBP, PPARα, or PPARγ, followed by qPCR quantification of PNPLA3 mRNA. Human PNPLA3 cDNA was isolated, and the I148M variant was generated by site-directed mutagenesis. Wild-type and I148M cDNAs were expressed in IHH and visualized by confocal microscopy. Expression of adiponutrin protein in human subcutaneous adipose tissue and liver was studied by Western blotting.
Results: PNPLA3 mRNA was induced by high glucose, dependent on ChREBP. The oxysterols 25- and 22(R)-hydroxycholesterol suppressed the mRNA, in the absence of effect by the LXR agonist T0901317, suggesting regulation of PNPLA3 by SREBP1c. Furthermore, stimulation of FXR by chenodeoxycholic acid or GW4064 suppressed PNPLA3. Both wild-type adiponutrin and the I148M variant were found to localize extensively on lipid droplets in IHH cells. Western analysis demonstrated abundant presence of adiponutrin protein in both human subcutaneous adipose tissue and liver.
Conclusion: The results are consistent with a function of adiponutrin associated with up-regulation of hepatic lipogenesis by carbohydrate feeding. PNPLA3 expression is positively controlled by central transcription factor systems responsive to glucose and insulin, ChREBP and SREBP1c, which enhance glycolysis and lipogenesis. PNPLA3 is suppressed by FXR, an effect possibly mediated via SREBP1c. Location of adiponutrin on lipid droplets suggests its intimate involvement in neutral lipid metabolism on the droplet surface.

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Discovery of a novel *in vivo* active heterocyclic inhibitor of stearoyl-CoA desaturase

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Background and aims: Stearoyl-CoA desaturase (SCD1) is linked to the pathogenesis of obesity, dyslipidemia and type 2 diabetes. SCD1 is rate-limiting in the synthesis of monounsaturated 16:1 n-7 and 18:1 n-9 fatty acyl-CoAs and catalyses an essential part of lipogenesis. Here, we describe the identification, *in vitro* properties and *in vivo* efficacy of a novel heterocyclic small molecule SCD1 inhibitor.
Materials and methods: SCD1, cytochrome b5 reductase, FADS1 and FADS2 activities were determined in rat liver microsomes. Cellular human

and rat enzyme activities were measured in HepG2 and H4IIE hepatoma cell lines, respectively. Metabolic stability was determined in liver microsomes, cell permeability in CaCo-2 cells. Male obese ZDF rats were used to assess *in vivo* effects on serum desaturation indices, body weight, blood glucose and triglycerides in a 4 weeks multiple dose study. All clinical blood parameters were determined by commercially available diagnostic kits on a Hitachi 912 device, serum fatty acid desaturation indices were analysed by LCMS.
Results: Hexahydropyrrolopyrole SCD1 inhibitors were discovered and a compound representative of the series was optimised to an IC50 of 7nM in rat liver microsomes and a cellular IC50 of 56 nM in rat H4IIE hepatoma cells. The compound is highly selective towards fatty acid desaturases 1 and 2 (D5 and 6 desaturases) and cytochrome b5 reductase (> 400-fold). Low metabolic lability in liver microsomal fractions (4%) and high cell permability ($401x10^7$ cm/s) allowed an *in vivo* assessment in ZDF rats. After 5 days dosing at 20mg/kg *per os* serum C_{max} levels reached 4520 ng/mL and a plasma half life of 14 hrs could be determined. After 28 days of treatment at 20mg/kg, the compound significantly decreased body weight (-8.1±0.5%), serum triglycerides (-56.1±4.0%), blood glucose levels (-28.0±3.4%) and HbA1c (-22.8±5.8 %) compared to vehicle in the obese animals. In parallel, serum fatty acid desaturation indices were decreased (-94.8±11.6%). However, fissures of the eye lid, alopecia and inflammation of the skin were observed from day 14 on in all animals treated with the same metabolically active dose.
Conclusion: In summary, we described *in vitro* and *in vivo* properties of a novel, potent and selective SCD1 inhibitor that improved body weight, blood glucose and triglycerides in an animal model of obesity, type 2 diabetes and dyslipidemia. However, the favourable *in vivo* features of systemic SCD1 inhibition shown in this study were accompanied by adverse target-related effects observed in skin. Thus, systemic SCD1 inhibition by small molecules might therefore not represent a feasible approach for the treatment of chronic metabolic diseases.

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Expression of nonalcoholic fatty liver disease associated adiponutrin variant I148M causes triglyceride accumulation
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Background and aims: The strong association between adiponutrin variant I148M and hepatic steatosis has recently highlighted adiponutrin as an important putative enzyme involved in lipid metabolism. Based on structural similarities to patatin domain-containing proteins, adiponutrin has been proposed to have both lipolytic and lipogenic capabilities, but its function and physiological relevance in lipid homeostasis are still unknown. Despite efforts, the biological role of adiponutrin and the mechanisms underlying the strong association between the I148M adiponutrin variant and elevated hepatic triacylglycerol levels remain elusive. Here we examine the impact of the polymorphism encoding I148M in adiponutrin on lipid storage in HEK293 cells.
Materials and methods: HEK293 cells were transfected with human wild type, I148M, C99G and K434G variants of adiponutrin. 36h post transfection the cells were analyzed with regard to lipid content, profile of extracted lipids and lipid accumulation by imaging of stained cells.
Results: Overexpression of wild type or the I148M variant of adiponutrin leads to significant increases in triglyceride content [30% ($p<0.05$) or 2-fold ($p<0.001$), respectively] compared to control transfected cells. Interestingly, the I148M variant caused a significantly greater [50% ($p<0.001$)] lipid accumulation compared to wild type adiponutrin. Thin layer chromatography of extracted lipids from parallel experiments confirmed that both wildtype and the I148M variant of adiponutrin cause accumulation of triacylglycerol in cells, leaving monoacylglycerol and diacylglycerol content unaffected, and that I148M more effectively promotes triglyceride formation compared to wild type. Imaging experiments of fixed cells revealed increased neutral lipids contained in visibly larger lipid droplets in wild type and I148M transfected cells compared to control. The control variants C99G and K434G behaved similarly to wild type adiponutrin in all experiments.
Conclusion: The presented data where expression of human recombinant adiponutrin promotes lipid storage suggests a primarily lipogenic rather than a lipolytic role for adiponutrin. Our data showing an increased lipogenic potential of the I148M variant of adiponutrin, compared to wild type, corroborates the strong association between I148M and liver steatosis in patients.
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Adipocyte control of cancer cell growth
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Obesity is one of the most challenging and growing health problems, worldwide. Epidemiologic studies now provide compelling evidence that obesity is a risk factor for both cancer incidence and mortality. In particular, obesity increases rates of breast cancer in postmenopausal women and is associated with a more poor survival and increased recurrence of disease, regardless the menopausal status. It is now becoming clear that adipocytes, which represent very abundant cell types surrounding cancer cells, particularly in the mammary gland, could influence several aspects of tumorigenesis, from promoting local invasion to angiogenesis and metastasis. However, the molecular mechanisms involved in the adipocyte control of the malignant phenotype remain poorly understood. We have studied the mechanisms by which adipocytes may affect breast cancer cell phenotypes. We have obtained evidence that conditioned medium (CM) of adipocytes derived from human mammary adipose tissue and from subcutaneous abdominal fat biopsies was capable to elicit growth of MCF7 breast cancer cells. This was also observed when mature adipocytes were obtained from undifferentiated precursors isolated from the stromal-vascular fraction (SVF). Similarly, CM from 3T3-L1 cells induced growth of MCF7 cells, in a time-dependent manner. In particular, CM from fully differentiated adipocytes was 2-fold more effective than CM from pre-adipocytes in inducing MCF7 growth. Cell cycle analysis by flow cytometry revealed that these changes are due to reduced apoptosis instead of increased proliferation. Multiplex screening for growth factors in the CM revealed that VEGF, FGF and PDGF secretion is higher by SVF cells than by adipocytes, suggesting a major involvement of SVF in promoting angiogenesis. In contrast, the content of IGF-1 produced by adipocytes is two-fold higher than that released by pre-adipocytes. Thus, IGF-1 could be a good candidate in mediating survival effect of adipocyte CM. Moreover, treatment of MCF7 with the IGF-1R inhibitor AG1024 reverted the adipocyte CM effect on cell growth. In conclusion, adipocyte-derived factors promote breast cancer cell growth by inhibiting apoptosis. This effect is more evident for factors released by adipocytes than by pre-adipocytes and is, at least in part, mediated by IGF-1.

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High dose detemir inhibits proliferation of a human bladder cancer T24 cell line independent of Akt and ERK pathway
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Background and aims: Growing evidence indicated the involvement of mitogenicity of insulin and insulin analogues in the increased risk of carcinogenesis in diabetes subjects. This study was aimed to investigate the effect of insulin detemir in comparison to regular human insulin on proliferation of a human bladder cancer T24 cell line and the role of Akt and ERK in the process.
Materials and methods: T24 cells were cultured and treated with detemir or insulin at the indicated concentrations for different time courses. A specific inhibitor for ERK1/2 (PD98059) or Akt (LY294002) was used either alone or in combination with detemir or insulin to test the involvement of Akt and ERK pathway in detemir or insulin-induced cell proliferation as evaluated using cell counting kit-8 reagents. Protein levels of Akt, pAkt, ERK, pERK were detected by Western blotting.
Results: Human insulin time- and dose-dependently enhanced proliferation of T24 cell at the concentrations of 10 and 100IU/L after treatment for 48, 72 and 96 hours. However, detemir inhibited T24 cell growth at 100IU/L. Both insulin and detemir promoted phosphorylation of ERK and Akt at 15, 30, and 60 min of treatment. Although ERK inhibition significantly enhanced while Akt inhibition reduced T24 cell proliferation, insulin still promoted while detemir inhibited T24 cell growth in the presence of either Akt or ERK inhibitor.

Conclusion: Insulin promotes proliferation of T24 cells. Detemir inhibits T24 cell proliferation at high dose independent of Akt or ERK activation.
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Anti-apoptotic activities of insulin and insulin-like growth factor (IGF) 1 in human osteosarcoma Saos-2/B10 cells
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Background and aims: Obesity, insulin resistance and hyperinsulinaemia are associated with an increased risk of growth progression of some cancer types and possibly also with enhanced survival of tumour cells. We studied anti-apoptotic effects of insulin and IGF 1 in a cell line which is critically dependent on IGF- or insulin- like signals for proliferation and survival in vitro.
Materials and methods: B10 cells were grown in serum-containing media and then exposed to serum-free test media. DNA synthesis was measured as 3H-thymidine incorporation during a pulse given 18-21 h following exposure to test media, and apoptosis was assessed after 4 h by an ELISA detecting cytosolic oligonucleosomes. Insulin/IGF 1 signalling was analysed by Western blotting.
Results: Effects of insulin and IGF 1 were dose-dependent: 0.4 nM IGF 1 or 20 nM insulin was required for half-maximal stimulation of DNA synthesis whereas 0.04 nM IGF 1 or 1 nM insulin resulted in half-maximal inhibition of apoptosis. Effects of insulin and IGF 1 were time-dependent: 1 nM IGF 1 or 100 nM insulin prevented apoptosis half-maximally when added for the last 1-2 (of a total of 4) h whereas sequestering IGF 1 (but not of insulin) by adding IGF-binding protein (IGFBP)-3 led to a half-maximal loss of protection from apoptosis within the last 1-2 (of 4) h. Insulin and IGF 1 both activated Akt/PKB to similar levels, but activation of ERK1/2 was higher in the presence of insulin (after stimulation for 4 h). Insulin or IGF 1 did not protect against apoptosis in the continuous presence of 100 nM wortmannin (4 h) which was associated with reduced phosphorylation of PKB but more than half-maximal protection was still found when wortmannin was added only for the last 3 h.
Conclusion: Continuous exposure to IGF 1 (suppressible by IGFBP-3) or to insulin (not suppressible by IGFBP-3) prevents B10 cell apoptosis, possibly dependent on Akt/PKB. Apoptosis appears to be more sensitive to regulation by IGF 1 and insulin than mitogenesis.

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The role of juxtaposed with another zinc finger gene 1 on glucose-lipid metabolism and related genes *in vitro*
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Background and aims: Epidemiologic studies have shown the association of Diabetes Mellitus (DM) with either increased or decreased risk of developing malignant tumors. Recently, a genome-wide association studies have suggested the association of JAZF1 (juxtaposed with another zinc finger gene 1) with DM and prostate cancer. JAZF1 encodes a 27 kDa nuclear protein containing three putative zinc finger motifs, and is expressed in a variety of tissues of mouse, with highest expression in adipose tissue and testis. however, little is known about the functions in regulating metabolism. In our study, we investigated the influence of an overexpression of JAZF1 on 3T3-L1 adipose cells and hepatoma carcinoma Hepa1-6 cells which represent target tissue for diabetes and insulin resistance.
Materials and methods: To survey the tissue distribution of JAZF1 in healthy C57BL/6J mice by real-time quantitative PCR(SYBR GreenI); Expression vector for JAZF1 gene was constructed and transient transfected into 3T3-L1 preadipocytes and hepatoma carcinoma Hepa1-6 cells, respectively. Oil Red O staining for 3T3-L1 preadipocytes was carried out. The mRNA levels of JAZF1, GLUT1,GLUT4, FAS(fatty acid synthetase), ACC(acetyl-CoA carboxylase), SREBP1(Sterol Regulatory Element Binding Protein1), and HSL(Hormone Sensitive lipase) implicated in glucose and lipid metabolism were determined by RT-QPCR; JAZF1 protein levels were measured by western blot.
Results: JAZF1 is expressed in all tissues examined, with highest levels detected in testis and adipose tissues and lowest levels in muscle and kidney. In JAZF1-transfected adipocytes, JAZF1 mRNA expression and protein levels were significantly higher than control cells after transfected 48h. The mRNA levels of HSL was increased obviously($P<0.05$)in JAZF1 transfection group compared with negative control and empty vector group, and the relative expression of FAS,ACC,SREBP1 mRNA were decreased significantly(all $P<0.001$). However, the mRNA levels of GLUT1,GLUT4 were not changed (all $P>0.05$). Intercelluar lipid accumulation was decreased obviously ($P<0.05$)by Oil Red O staining and colorimetric in JAZF1-transfected cells compared with negative control and empty vector group.
Conclusion: There were a widely expression of JAZF1 in various organization of C57BL/6J mice, it indicated that JAZF1 might play a role in maintaining normal physiological function. These results show that overexpression of JAZF1 in 3T3-L1 cells can reduce lipid synthesis, increase lipolysis and improve lipid accumulation. We speculate that JAZF1 might provide a new potential therapeutic target for obesity and diabetes.
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Induction of HSP72, a potential novel therapeutic approach for metabolic syndrome and type 2 diabetes

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Background and aims: Life-style related diseases, such as type 2 diabetes and metabolic syndrome (MS) are associated with reduction of heat shock protein (Hsp) 72 levels, and activation of Hsp72 expression may improve insulin resistance and visceral adiposity. The present study investigated whether Hsp72 induction using an apparatus which provides Mild Electrical stimulation with Thermotherapy (MET) could reduce visceral adiposity and improve glucose homeostasis in mice models of type 2 diabetes and in human with MS.
Materials and methods: C57/BL6 mice fed with high-fat feeding and db/db mice were treated with MET (12V, 55 pulses per second, 10 min at 42°C, twice a week for 8~10 weeks) or sham-treatment. High-fat fed mice were also treated with Hsp72 inducer geranylgeranylacetone (GGA), 200 mg/day for 4 weeks. Forty eligible male MS subjects were randomly assigned to two groups, each containing 20 subjects. Group I was subjected to a 12-week intervention period of MET (12V, 55 pps, 60 min at 42°C, 4 times a week) followed by 12 weeks with no treatment. The order was reversed in Group II.
Results: High-fat fed mice and db/db mice presented following favorable changes upon treatment with either MET or GGA administration. 1) Reduction of visceral (-34%) and subcutaneous (-44%) fat mass, 2) Reduction of fasting glucose (-20%) and insulin levels (-38%), 3) Improvement of glucose homeostasis and insulin resistance, 4) Upregulation of serum adiponectin levels (+94%), 5) Improvement of insulin signaling in liver. We observed following results upon MET treatment in male subjects with MS. 1) Reduction of visceral fat (-5.8%) and total abdominal fat (-3.3%) area, but not subcutaneous fat area, 2) Decrease of waist circumference (-0.7%), but not body weight, 3) Reduction of both systolic (-4.2%) and diastolic blood pressure (-2.7%), 3) Improvement of fasting plasma glucose (-4.9%) and insulin levels (-8.5%) as well as HOMA-IR (-11.7%), QUICKI (+2.4%) and composite WBISI (+17.2%), 4) Trend of reduction in HbA1c (-1.2%, p=0.065), 5) Decrease of insulinogenic index (-10.2%), 6) Amelioration of inflammatory cytokines or adpokines, such as hs-CRP (-27.4%), adiponectin (+9.3%), leptin (-8.3%) and TNF-α (-10.2%), 7) Decrease of WBC (-5.4%) and LDL-C (-5.3%) levels.
Conclusion: Hyperthermia with mild electrical stimulation improved glucose homeostasis and insulin resistance in mice models of type 2 diabetes and human with MS. Therefore, this treatment could have beneficial impacts on body composition and metabolic abnormalities in life-style related diseases. Type 2 diabetes could be one of the other targets for the treatment.
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Taurocholate delivered to the distal gut suppresses food intake and causes weight loss in rats

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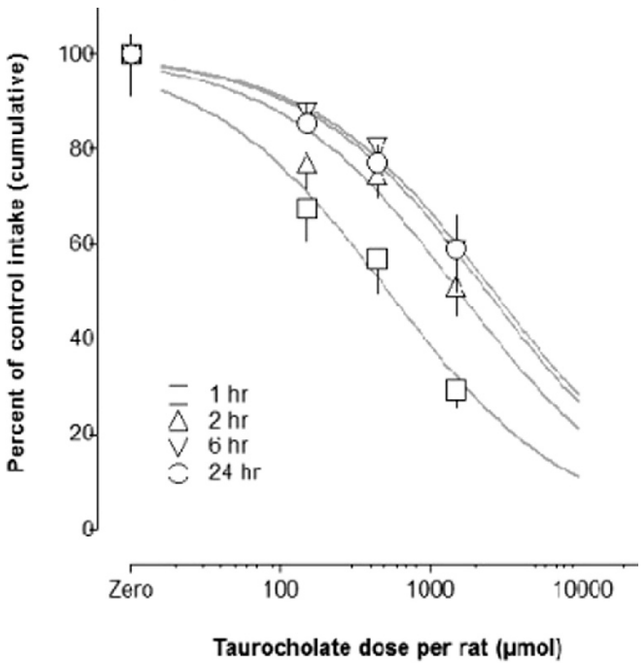
Background and aims: Bile acids (BA's), the endogenous ligands for TGR5 receptors located on enteroendocrine L-cells, stimulate the secretion of the anorexigenic hormones GLP-1, PYY and oxyntomodulin, as well as GLP 2. Several orally administered TGR5 agonists do not evoke robust anorexic effects, perhaps by failing to reach colon and rectum, wherein the peptide content and secretory response of L cells can be over 2 orders of magnitude higher than in more accessible stomach and duodenum.
Materials and methods: Effects of rectal BA's on food intake were studied in [male] Sprague Dawley rats fasted 12 hours. In a series of dose-response experiments, each of 12 rats received each of 4 taurocholate (TCA) doses 0 (control), 0.15, 0.45 or 1.5 mmol in 3 replicates of a 4x4 Latin Square design. In separate study, taurocholate was delivered to the distal gut of [male] Sprague Dawley rats via an intraperitoneally implanted osmotic minipump (Alza) and intraluminal catheter at a rate of 6 µmol/hour (n=6) for 7days; control group minipump delivered saline only (n=6).
Results: Cumulative food intake measured up to 24 hours after rectal delivery of TCA was dose dependently decreased. Relative to controls, food intake in TCA treated rats was reduced by 32%, 43%, and 70% by 0.15, 0.45 and 1.5 mmol doses, respectively (P<0.05, 1-way ANOVA). The ED50 for TCA-induced reduction in cumulative food intake at 1 hour was 1.6 mmol. For 24 hour intake, the value was 7.2 mmol. In the second study 10% weight loss induced by taurocholate delivered for 7days was greater than the 5% observed in surgical controls (P<0.05).
Conclusion: In conclusion, we have demonstrated an effect of rectally administered taurocholate to dose-dependently inhibit food intake in normal rats for up to 24 hours, and for sustained taurocholate delivery to the lower bowel to induce weight loss.



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Weight loss during a hypo-caloric diet induces an anti-inflammatory response in adipose tissue

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Adipose tissue of obese insulin resistant subjects is characterized by a low-grade inflammatory phenotype and reduced levels of adiponectin and GLUT4. The low-grade inflammation is associated with pro-inflammatory macrophages in adipose tissue. The aim of this study was to evaluate whether this inflammatory response is an early or late feature during the course of weight gain. Therefore, we studied the inflammatory changes in plasma and subcutaneous adipose tissue in healthy lean men before and after a positive energy balance resulting in modest weight gain, and a negative energy balance resulting in loss of the gained weight. We studied 9 healthy lean men (age 37 [27-43] years and BMI 23.6 [20.6-25.6] kg/m^2). The hypercaloric diet was calculated as 1.4 x caloric need to maintain body weight. After a median of 35 [28-43] days participants gained 7 [5.1-7.6]% of their initial body weight. The hypocaloric diet was calculated as 1.00x resting energy expenditure. At the end of the hypo-caloric diet participants returned to their initial weight. The protein, fat and carbohydrate content of the diets were 16%, 30% and 54% respectively. Participants were monitored weekly to assess body weight, body composition, and plasma leptin concentrations. Before the diet intervention and after the hypercaloric (HYPER) and hypocaloric (HYPO) diet, blood samples and abdominal subcutaneous adipose tissue (AT) biopsies were taken after an overnight fast to measure plasma concentrations and AT expression levels of inflammatory markers and adipokines. Plasma levels of leptin, adiponectin and MCP-1 were all increased in the HYPER state. In AT, inflammatory markers (TNF, IL10, MCP1, osteopontin) did not change significantly, but expression of the mannose receptor (MR) decreased significantly. Interestingly, expression of GLUT 4 in AT was significantly increased during the posi-

tive energy balance. During the period of the negative energy balance, plasma adiponectin, MCP-1 levels and resistin returned to baseline, while leptin levels decreased below baseline levels. In AT expression of adiponectin and a trend for decreased GLUT4 expression compared to baseline was observed. Interestingly, the expression of CD68, CD163 and MR increased in the HYPO state, indicating that there was a higher content of alternatively activated macrophages after the negative energy balance. In conclusion, in the early phase of a positive energy balance resulting in modest weight gain, a limited pro-inflammatory response in plasma is present. GLUT 4 expression is increased facilitating triglyceride formation. During a negative energy balance, alternatively activated macrophages are present in AT indicating an anti-inflammatory response which may be important in tissue remodelling.

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Phentermine/topiramate combination therapy significantly improves glucose impairment in overweight/obese patients

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Background and aims: Obesity, which is strongly linked to type 2 diabetes mellitus (T2DM), is on the rise. Impaired fasting glucose/impaired glucose tolerance (IFG/IGT) are prediabetic glycemic states associated with insulin resistance and increased risk of cardiovascular disease. A 56-week Phase 3 study (CONQUER) was performed to confirm the efficacy and safety of once-daily, low-dose, controlled-release phentermine/topiramate (PHEN/TPM CR) in overweight/obese patients with at least 2 comorbidities such as prediabetes or T2DM. In addition, this study evaluated the effects of PHEN/TPM CR on various glycemic parameters in a subgroup of patients with IFG/IGT.
Materials and methods: All subjects underwent oral glucose tolerance testing (OGTT) at baseline, Week 4, and Week 56. From this, a subgroup of subjects with IFG/IGT at baseline was defined using fasting serum glucose >5.55 mmol/L, or serum glucose >7.77 mmol/L 2 hours after a 75 g glucose load.
Results: Out of 2487 patients enrolled, 1635 (66%) were identified as having IFG/IGT. Patients were randomized to either placebo (n=655), PHEN/TPM CR 7.5/46 mg (7.5/46 [n=335]), or PHEN/TPM CR 15/92 mg (15/92 [n=645]); treatment with both doses of PHEN/TPM CR resulted in a significantly greater percent weight loss than placebo. For subjects with IGT/IFG, the mean percent weight loss at Week 56 with LOCF was 2.3% for placebo, 8.3% for 7.5/46, and 10.5% for 15/92. The table presents the results for change in HbA1c and fasting serum glucose from baseline to Week 56 (ITT-LOCF). Both PHEN/TPM CR doses led to significantly greater improvement vs placebo in HbA1c and fasting serum glucose. Further analysis showed that, among the subgroup of patients with IFG/IGT (per OGTT) who completed the study (n=562), achievement of normal OGTT was 44.2%, 63.1%, and 73.1% for placebo, 7.5/46, and 15/92, respectively (P<0.0001 vs placebo) (Table). The percentage of IFG/IGT subjects progressing to T2DM during the study was 15.3%, 6.9%, and 4.5% for placebo, 7.5/46, and 15/92, respectively. PHEN/TPM CR was well tolerated.
Conclusion: In addition to achieving significant weight loss, patients with IFG/IGT who were treated with PHEN/TPM CR achieved significant improvements in glycemic parameters and decreased incidence of progression to worsening glycemic status, suggesting prevention of progression to diabetes and/or reversion to normal glycemic status.

Change in Glycemic Parameters From Baseline to Week 56 in Subjects With Impaired Glucose (ITT-LOCF)

Parameter	Placebo	PHEN/TPM CR 7.5/46	PHEN/TPM CR 15/92
HbA1c (%)			
n	539	309	594
LS Mean	0.0	-0.1*	-0.2*
Fasting Serum Glucose (mmol/L)			
n	628	328	623
LS Mean	-0.16	-0.34*	-0.42*
Achievement of normal OGTT			
n	190	69	145
Percentage of patients	44.2	63.1**	73.1*

*P<0.0001 vs placebo **P=0.0005 vs placebo

Supported by: Vivus, Inc

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Once-daily, low-dose, controlled-release phentermine/topiramate results in significant clinical improvements in overweight/obese patients with type 2 diabetes

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Background and aims: Weight loss can delay progression to type 2 diabetes mellitus (T2DM) and improve glycemic control in patients with T2DM. A 56-week Phase 3 trial (CONQUER) was conducted to evaluate efficacy and safety of low-dose, controlled-release phentermine/topiramate (PHEN/TPM CR) for weight loss in overweight/obese patients with and without T2DM, and effects on glycemic control and medication load in T2DM patients.
Materials and methods: In this randomized, double-blind trial, 2487 obese subjects meeting at least 2 criteria for the metabolic syndrome received placebo (n=994), PHEN 7.5 mg/TPM CR 46 mg (7.5/46 [n=498]), or PHEN 15 mg/TPM CR 92 mg (15/92 [n=995]). At baseline, T2DM subjects were treated with lifestyle intervention or metformin; they were managed to ADA standards of care. This T2DM subpopulation was further analyzed.
Results: At baseline, the mean weight of all randomized subjects was 103.1 kg, mean BMI was 36.6 kg/m^2, and mean HbA1c was 5.9%. In total, 338 (15.8%) patients had a previous diagnosis of T2DM or an elevated fasting glucose (FG) (>6.94 mmol/L) at baseline. Mean baseline HbA1c for the T2DM subpopulation was 6.8%; mean FG was 7.43 mmol/L. At Week 56, the least squares (LS) mean weight loss for placebo, 7.5/46, and 15/92, was 1.2%, 7.8%, and 9.8%, respectively, for the overall ITT-LOCF population (P<0.0001 for PHEN/TPM CR vs placebo). Those subjects completing 56 weeks of treatment on drug also experienced significant weight loss vs placebo (P<0.001): 1.6%, 9.6%, and 12.4% for placebo, 7.5/46, and 15/92, respectively. Among ITT-LOCF subjects with T2DM at baseline, LS mean weight loss was significant vs placebo at Week 56 (P<0.0001): 1.9%, 6.8%, and 8.8% for placebo, 7.5/46, and 15/92, respectively. During the study, a greater proportion of subjects with T2DM in the placebo group required an increase in concomitant antidiabetic medications than those in the PHEN/TPM CR groups (Table). LS mean decrease in HbA1c from baseline to Week 56 in subjects with T2DM (ITT-LOCF) was significantly greater with both doses of PHEN/TPM CR vs placebo (0.4%, 0.4% vs 0.1%; P<0.05). LS mean decreases from baseline in FG (mmol/L) were also greater in the PHEN/TPM CR groups vs placebo: 0.31, 0.54, and 0.66 for placebo, 7.5/46, and 15/92, respectively (P=0.0556 for 15/92 versus placebo; P=0.3325 for 7.5/46 vs placebo). PHEN/TPM CR was well tolerated with the majority of adverse events (AEs) being mild in severity. The most common AEs were tingling, dry mouth, constipation, altered taste, and insomnia.
Conclusion: In this study, patients with T2DM experienced significant weight loss with PHEN/TPM CR therapy. These results were associated with clinically meaningful improvements in glycemia through 56 weeks compared to placebo, despite increased use of diabetes medications in the placebo group. Thus, PHEN/TPM CR can enhance glycemic control in overweight/obese patients with T2DM.

Changes in Number of Antidiabetic Medications, Baseline to Week 56: Diabetic Population (ITT-LOCF)

Treatment	n	Subjects With Decrease n (%)	Subjects With No Change n (%)	Subjects With Increase n (%)
Placebo	157	4(2.5)	130 (82.8)	23 (14.6)
PHEN/TPM CR 7.5/46	67	2 (3.0)	62 (92.5)	3 (4.5)
PHEN/TPM CR 15/92	164	6 (3.7)	151 (92.1)	7 (4.3)

Supported by: Vivus, Inc

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Restoration of beta cell function in severely obese type 2 diabetic patients after gastric bypass surgery

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Background and aims: Gastric bypass surgery has been shown to frequently resolve type 2 diabetes but the mechanism underlying are incompletely understood. Here we report preliminary results on a systematic assessment of beta-cell function along with insulin sensitivity before and shortly after gastric bypass surgery in the first 6 type 2 diabetic patients of an ongoing study.
Materials and methods: Before and 8 to 21 days after the operation, patients were subjected to an OGTT as well as to a botnia clamp which combines an IVGTT with a subsequent hyperinsulinemic-euglycemic clamp. Established models were used to calculate varies indices of glucose metabolism. Known diabetes duration in our patients ranged from 1 to 14 years (mean ± SEM: 8.2 ± 2.0 years).
Results: Body weight decreased from 121.4 ± 9.2 to 113.7 ± 8.4 kg and BMI from 43.3 ± 1.5 to 40.7 ± 1.6 kg/m^2 (both $P < 0.005$) and diabetes medication was markedly reduced after the operation (before: 4 patients short- and long-acting insulin, average dose 56.5 ± 12.1 U/day and 51.5 ± 10.0 U/day, respectively, 6 patients metformin, one patients gliclazide; after: 4 patients long-acting insulin, average dose 21.5 ± 5.9 U/day, no short-acting insulin, no oral antihyperglycemic agents). Concentrations of HbA1c, fasting glucose, and 2 h glucose response to the OGTT were significantly reduced after surgery (all $P < 0.05$). None of the calculated indices of insulin secretion, e.g. acute insulin response (AIR), pointed to a significant improvement of beta-cell function (all $P > 0.5$) while indices of insulin sensitivity tended to increase (e.g. M-value 1.65 ± 0.59 vs. 3.67 ± 0.52; $P = 0.07$).
Conclusion: In contrast to previous studies in type 2 diabetic patients not treated with insulin with disease duration less than 6 years, we did not find an improvement in beta-cell function shortly after gastric bypass surgery. Thus, improved glucose metabolism soon after the operation appears to rely primarily on enhanced insulin sensitivity. However, these preliminary observations do not exclude a restoration of beta-cell function later after gastric bypass surgery.
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Sleeve gastrectomy, only a restrictive surgical procedure?

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Background and aims: Bariatric Surgery (BS) offers new therapeutic possibilities for subjects with morbid obesity (MO) and type 2 diabetes mellitus (DM2). Several studies suggest that the malabsorptive surgical procedures are more effective than restrictive techniques (Gastric Banding and Vertical Banded Gastroplasty) for the treatment of DM2. The aim of this study is to assess the efficiency of Laparoscopic Sleeve Gastrectomy (LSG) vs Laparoscopic Y de Roux Gastric By-pass (LYRGB) in the normalization of glucose metabolism disorders, and to analyze subsequent changes in Insulin Resistance (IR) in patients with and without glucose metabolism abnormalities.
Materials and methods: Cohort study of MO patients consecutively admitted for BS (LYRLB or LSG). Patients were grouped according to glucose metabolism state categories defined by ADA: DM2, impaired fasting glucose (IFG) and normal glucose metabolism (nonDM). IR was defined as a Homeostasis model assessment (HOMA-IR) ≥3.29 (Spanish population study). The following clinical and metabolic parameters were gathered at baseline, 3, 6 and 12 months after the procedure: venous blood glucose, HbA1c, HOMA-IR and estimated glucose disposal rate (EGDR). DM2 remission was defined as: 2 consecutive glucose measurements <126mg/dl, HbA1c <6.5% and no need for hypoglycemic drugs. IFG normalization was defined as: 2 consecutive glucose values <100mg/dl and HbA1c <5.7%. And IR resolution was characterized by a HOMA <3.28. Multivariate analysis were used to identify predictive factors for remission of glucose metabolism disorders and IR.
Results: 140 patients were included with a mean age of 45.6 ± 7.9, 85.6 % women, and mean BMI of 45.7 ± 5.0 Kg/m2. 67.9 % underwent a LYRGB and 32.1 % a LSG. Baseline characteristics of DM2 patients in both surgical interventions were comparable in terms of age, sex, need of hypoglycemic drugs, DM2 duration, glucose levels and HbA1c. Clinical outcomes at three months follow-up in both surgical groups were as follows: 1) 80.6% DM2 remission, 2) 89.5% IFG remission, 3) IR remission was achieved in 79.2 % of the DM2 patients, in 95.9 % of IFG subjects and in 97.7 % of the nonDM subjects. Independent predictors for a significant decrease in HOMA-IR at 12 months were EGDR and BMI (R^2=0.449) . No predictive factors for DM2 or IFG resolution were identified.
Conclusion: A restrictive surgical procedure like LSG is equally effective as malabsortive techniques in terms of weight loss, improvement of glucose metabolism and IR. EGDR negatively correlates with IR improvement after the BS.

	LSG	LYRGB	significance
Age (years)	44.4 ± 9.8	46.1 ± 8.2	p>0.05
BMI (Kg/m^2)	44.5 ± 45.5	46.3 ± 4.7	p>0.05
EGDR (mg/kg^{-1} min^{-1})	8,9 ± 2.1	8,2±2.4	p>0.05
HOMA-IR	4.2 ± 2.4	4.6 ± 3.0	p>0.05
DM2/ IFG (%)	15.6 / 44.4	26.3 / 40.0	p>0.05
IR (%)	67.6	67.1	p>0.05
Percentage of excess weight loss 12 months	82.9 ± 18.8	80.9 ± 16.6	p>0.05
HOMA-IR decrease 12 months	2.2 ± 1.5	3.6 ± 3.0	p>0.05
IR remission (%) 12 months	95.5	100	p>0.05
IFG remission (%) 12 months	85	94.6	p>0.05
DM2 remission (%) 12 months	100	92	p>0.05

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Respiratory function in massive obesity: effects of surgically induced weight loss

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Background and aims: In obese patients, adipose tissue around the rib cage and abdomen and in the visceral cavity loads respiratory system, increases work of breathing and reduces lung volumes particularly functional residual capacity (FRC) and expiratory reserve volume (ERV). Obesity also affects airway caliber with a slight reduction in expiratory flows. To date, limited data are available on the effects of bariatric surgery on respiratory function. We aimed to systematically investigate respiratory function of obese patients at baseline (T0) and 6 months (T6) after surgically induced weight loss.
Materials and methods: We conducted a retrospective analysis of a single center observational cohort of 77 obese patients (BMI = 47.8 ±6.7 kg/m^2) who underwent laparoscopic bariatric surgery (63 Roux-en-Y-gastric bypass; 14 gastroplasty). Arterial blood gazes and respiratory function tests including lung volume and flow measurements were assessed at T0 and T6.
Results: Patient's characteristics at baseline were as follows: 74% women, mean age = 43 ± 11 years, mean PaO2 = 82.9 ± 9.2 mmHg, mean PaCO2= 40.2 ± 6.2 mmHg. Mean values of forced expiratory volume in 1 second (FEV_1), vital capacity (VC), FEV_1/VC and total lung capacity (TLC) as expressed in % of predicted values were in the normal range. FRC and ERV were significantly reduced (absolute values and % of predicted: 2.0 ± 0.6L; 65 ± 16% and 0.53 ± 0.33L; 44 ± 25% respectively). On flow volume loops, expiratory flows at 25% of VC was also decreased (3.5 ± 0.9L/min; 52 ± 26%). After bariatric surgery (T6), we observed a significant weight loss (Δ-30.6 ± 13kg; -22.4 % of the initial BMI, p<0.0001) along with a significant improvement from baseline in VRE (absolute values and % of variation from baseline: Δ0.4 ± 0.3L; +44 ± 95%; p<0.0001), expiratory flows at 25% of VC (Δ0.13 ±0.39L/min; +9 ± 33%; p=0.03), FRC (Δ0.4 ± 0.4L; +16 ± 18%; p<0.0001), FEV_1 (Δ0.2 ±0.2L/min; +7 ± 9%; p=0.004) and FVC (Δ0.2 ± 0.3L, +6 ± 8%, p=0.007). Moreover, we found an inverse association between BMI reduction and increases in ERV, expiratory flows at 25% and 50% of VC (p=0.0035; p=0.037; p=0.04, respectively). Changes in PaO2 and PaCO2 were close to a statistically significant improvement (ΔPaO2= 7.3 ± 22 mmHg; p=0.06, ΔPaCO2= -3.9 ± 9

mmHg; p=0.07). Whereas 25/77 patients (32.5%) were hypoxemic at baseline (PaO2<80mmHg), 13 improved with weight loss at 6 months. All of the 4 being hypercapnic at baseline normalized their PaCO2.
Conclusion: To our knowledge, this is the largest cohort evaluated in terms of respiratory function before and 6 months after surgically induced weight loss. Morbidly obese patients exhibited reduced expiratory reserve volume with increased risk of airway closure demonstrated by significant reduction of expiratory flows at 25% of VC. This is associated with underventilation of some lung regions explaining low PaO2 in more than 30% of our cohort. Importantly, surgically induced weight loss allowed a significant improvement in pulmonary functions, volumes, flows and blood gazes.

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Massive body mass loss leads to reduced endoplasmic reticulum stress and activation of autophagy in adipose tissue
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Background and aims: Endoplasmic reticulum stress (ER-stress) has emerged as an important link between nutritional overload and insulin resistance. High consumption of nutrients, especially saturated fats, induces the activation of ER-stress, which promotes inflammatory gene transcription and eventually the activation of pro-apoptotic signaling. The inflammatory proteins induced by this process mediate the activation of serine/threonine kinases in insulin sensitive tissues, contributing for the impairment of the insulin signal transduction. Body mass loss is one of the most efficient means for correcting glucose intolerance. The reduction of the adipose tissue production of inflammatory factors plays a central role in this process. However, the molecular and cellular mechanisms behind this outcome are poorly understood. Here, we explore the hypothesis that massive body mass loss is accompanied by reduced ER-stress in the adipose tissue.
Materials and methods: Ten obese patients were submitted to subcutaneous adipose tissue biopsy before and 6-8 months after bariatric surgery. Ten, age matched lean subjects were employed as controls. Samples were used for evaluation of protein and mRNA expression of markers of ER-stress, inflammation and autophagy. Metabolic parameters were evaluated at the same time-points as biopsies.
Results: Body mass index dropped from 48±4 to 37±4 (controls, 24±2), which was accompanied by the reduction of blood insulin, leptin, TNF-α, IL-6 and C-reactive protein and by the increase in the blood levels of adiponectin. Significant reductions in protein (amount/activity) and/or mRNA levels of the ER-markers, PERK, eIF2α, IRE1α, spliced XBP1 and JNK were observed. The inflammatory proteins TNFα, IL-1β, IL-6, IKK and SOCS-3 were also negatively modulated by body mass loss. Interestingly, markers of autophagy, such as beclin, LC3 and CHOP increased after body mass loss.
Conclusion: The reduction of ER-stress may be an important molecular/cellular mechanism linking the loss of body mass with reduced adipose-tissue-driven inflammation. The induction of autophagy in this setting may contribute to prolonged adipocyte survival during catabolism and may play an important role in body mass regain, which is commonly seen in patients undergoing restrictive dieting programs.
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Identification of determinants for weight reduction in children and adolescents with overweight and obesity with an standardised questionnaire and electronic health technology
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Background and aims: The prevalence of overweight/obesity increased markedly during the last decades. It is associated with a high risk for diabetes and death. Patients often fail to reach sufficient long-term weight reduction. The aim of the trial is the development of an research programme to identify the determinants of overweight/obesity.
Materials and methods: 97/117 children/adolescents with overweight/obesity admitted to our hospital to participate in a structured treatment and teaching programme (STTP) due to overweight/obesity were included in the trial (age 13.4±2.6 years, BMI 31.2±5.0 kg/m², BMI-SDS 2.49±0.52). All children filled out a standardized questionnaire and participate in an intelligence test. To asses physical activity and eating habits electronic health technology was used (Fraunhofer-Institute, IGD, Germany). The system consists in a motion sensor integrated in a mobile phone (DiaTrace). The system analyses kind, intensity & duration of physical activity and eating habits.
Results: During participation in the STTP the children/adolescents had a weight reduction of 6.97±2.91 kg (p<0.001). BMI/BMI-SDS decreased from 31.3±5.2 kg/m²/2.50±0.50 to 28.7±4.9 kg/m² (p<0.001)/2.15±0.57 (p<0.001). The IQ was 99.6±12.9 points for the vocabulary test & 96.4 ±15.3 points for the analytical test. There were significant correlations between weight reduction and body weight at onset of the trial (r=-0.564, p<0.001), family climate (r=0.265, p=0.046), stress management (r=0.273, p=0.04), eating behaviour (r=-0.272, p=0.001), intrinsic motivation (ß=0.732, p<0.001), intrafamilial conflicts (ß=0.461, p=0.04), introspection (r=-0.331, p=0.012) duration of physical activity measured with MoSeBo/DiaTrace (ß=-0.438, p=0.002) and body fat mass (ß=-0.393, p=0.005). Multivariate analysis shows associations with the motivation for weight reduction (ß= 0.388, p=0.021), conflicts at school (ß= -0.299, p=0.036), self-regulation of emotions (ß= 0.575, p<0,001), education of the father (ß= -0.246, p=0.039) and physical activity assessed with DiaTrace (ß=-0.181, p=0.044). Comparing self-reported physical activity with objective assessment by the diatrace system there were significant differences (p<0.001). In general the duration of physical activity documented by children and adolescents was much higher than the objective assessment. Similarly, the real caloric intake was higher than recommended (p=0.085).
Conclusion: 1. There are important psychological parameters with a association with the weight reduction. On the background of the identified parameters a new questionnaire will be developed and used in a larger multicenter cohort. In a 1 year follow-up examination all determinants for weight reduction will be evaluate. After that a systematic adaptation of the STTP should follow. 2. There are differences between patients´ self-assessment and objective perception of physical activity and eating habits. Discrepancies in perception is a important determinants of poor long-term outcome. Using modern electronic health technology allows the objective assessment of kind, intensity and quantity of physical activity and eating habits. This could be an important advanced method to improve the therapy of obesity and diabetes.

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Fatty acid oxidation rate is higher in obese women than obese men

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Background and aims: Excess plasma fatty acids have been associated with insulin resistance, increased hepatic triglyceride production and cardiovascular risk. Women have been shown to produce more fatty acids than men relative to their resting energy expenditure. This study investigated whether the oxidation rate of circulating fatty acids is also greater relative to resting energy expenditure in obese women compared to men matched for age and BMI.
Materials and methods: 12 obese men (58 ± 2y, BMI 31 ± 1 kg/m^2)(mean ± SEM) and 12 postmenopausal obese women (59 ± 1y, BMI 32 ± 1 kg/m^2) were studied. Resting energy expenditure (REE) and total lipid oxidation was measured by indirect calorimetry. An iv infusion of [U-^{13}C] palmitate was administered with measurements of plasma palmitate enrichment and $^{13}CO_2$ production rate to calculate palmitate production, metabolic clearance (MCR) and oxidation rate. On a separate day an iv infusion of [1,2-^{13}C]acetate was given with measurements of $^{13}CO_2$ production rate to correct palmitate oxidation for the loss of label in the Krebs cycle. Whole body fat mass was measured by MRI. Statistical analysis was by unpaired t test.
Results: Palmitate turnover rate (Ra), MCR and oxidation rate (Ox) expressed as kg fat free mass (FFM) were significantly higher in obese women than in obese men (Ra: 4.5 ± 0.3 v 2.8 ± 0.2 umol/min/kgFFM, p<0.001); MCR 31.2 ± 1.5 v 20.7 ± 1.4 ml/min/kgFFM, p<0.001; Ox 1.6 ± 0.1 v 1.2 ± 0.1 umol/min/kgFFM, p<0.002). Palmitate Ra expressed as kg fat mass was not significantly different between genders. Plasma palmitate concentrations and total lipid oxidation expressed as kg FFM, measured by indirect calorimetry, were not different in the women and men. When corrected for REE both palmitate Ra, MCR and Ox remained significantly greater in women than men (p<0.003, p=0.006, p=0.03 respectively). When subset of subjects (7men, 7 women) were matched for adiposity (men: 38 ± 3 kg fat, women 38 ± 1 kg fat), these measurements remained significantly greater in women than in men (p=0.003, p<0.01 and p=0.002 respectively).
Conclusion: This study shows that when adjusted for FFM and REE, palmitate oxidation rate was higher in women than men suggesting that women oxidise more circulating fatty acids than men. Since total lipid oxidation adjusted for FFM did not differ between men and women this suggests that men oxidise more non-plasma lipids.
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Metabolomics reveals differential metabolic regulation at the catabolic-anabolic switchpoint during oral glucose challenge testing in women after recent gestational diabetes

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Background and aims: Insulin-mediated postprandial suppression of non-esterified fatty acid release from adipose tissue is acknowledged as an important physiological function to protect non-adipose tissues from lipotoxic effects. Functional metabolic characterization of the catabolic-anabolic transition in the early postprandial state is supposed to provide valuable insight into. The PINGUIN trial is a randomized intervention trial assessing the protective potential of Vildagliptin medication for the prevention of diabetes type 2 in a high-risk population of women after recent gestational diabetes. Extensive follow-up and longitudinal monitoring by repeated oral glucose and food challenge testing provides a unique opportunity to systematically analyze changes in metabolic systems performance during diabetes progression.
Materials and methods: After overnight fasting, eligible women and volunteers completed oral glucose tolerance testing or consumed a standard breakfast meal. Serum samples were drawn at 6-8 times between 20 min before and 120 min after the oral food challenge. Direct infusion- and HPLC-tandem mass spectrometry were used to quantitate amino acids, acylcarnitines, hexoses, sphingomyelins, phosphocholines and lysophosphocholines (180 metabolites in total) from serum sample time series.
Results: Targeted metabolomics analyses were able to show consistent responses of distinct metabolite groups rapidly following oral glucose or food uptake. Most notable changes occurred in short-chain acylcarnitines that represent the decreasing production and utilization of ketone bodies and organic acids. Similarly, levels of medium- and long-chain acylcarnitines and distinct long-chain sphingomyelins dropped rapidly, representing the suppression of fatty acid release from adipose tissue. Reaction profiles of amino acids were divided, prominent postprandial decrease was seen in all branched-chain and urea cycle-related amino acids, mirroring the halting of protein catabolism. Interestingly, fasting levels of several closely related acylcarnitines and amino acids were strikingly different in women with recent history of gestational diabetes as compared to healthy young volunteers. Some of the women showed differing metabolite pattern without significant changes over time, in contrast to the general decreasing trend. The significance of this finding has to be further evaluated by extending the study to include more subjects.
Conclusion: Assessment of dynamic metabolite changes during oral challenge testing reveals consistent pattern of metabolic regulation. Functional phenotyping by metabolomics techniques may be able to improve stratification of preventive intervention in populations at high risk for diabetes and metabolic syndrome.
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Serum concentrations and tissue expression of components of insulin-like growth factor-axis in patients with type 2 diabetes mellitus and obesity: the influence of very low calorie diet

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Background and aims: Insulin-like growth factor (IGF)-axis plays a complex role in glucose homeostasis, insulin sensitivity and pathogenesis of diabetes mellitus. The aim of the present study was to measure serum levels and tissue expression of selected components of the IGF-axis in type 2 diabetic patients at baseline and after dietary intervention.
Materials and methods: 12 obese females with type 2 diabetes mellitus (T2DM) and 10 healthy lean, sex- and age-matched controls (C) were included into the study. Serum concentrations of selected biochemical and hormonal parameters were measured by commercial ELISA and RIA kits. The expression analysis of genes for IGF-I and -II, insulin-like growth factor binding protein(IGFBP)-1 and -3 and insulin-like growth factor-receptor (IGF-R) in subcutaneous adipose tissue (SCAT) and isolated peripheral monocytes was performed by RT PCR at baseline and after 2 weeks of very low calorie diet (VLCD, energy intake 2500 kJ/day). The study was approved by the Ethical Committee of General University Hospital in Prague.
Results: Compared to C group, T2DM group had significantly increased fasting glucose, insulin and leptin concentrations and mRNA expression of IGF-R and IGFBP-3 in peripheral monocytes. Serum levels of IGF-I and adiponectin and mRNA expression of IGF-I, IGFBP-3 and IGF-R in SCAT were significantly reduced in T2DM group. IGF-II expression did not differ between the groups. mRNA expression of IGFBP-1 was not detected in either SCAT or peripheral monocytes. mRNA expression of IGF-I and IGF-II was not detected in peripheral monocytes. In SCAT, the mRNA expression of IGF-I, IGFBP-3 and IGF-R negatively correlated with BMI, insulin, glucose and HOMA index. IGF-R mRNA expression in peripheral monocytes positively correlated with BMI, insulin and HOMA index, while IGFBP-3 mRNA expression positively correlated only with BMI. Two weeks of VLCD significantly decreased body weight, and improved glycemia, insulin resistance and lipid profile. VLCD further significantly decreased serum IGF-I levels and increased IGF-I mRNA expression in SCAT. mRNA expression of other studied parameters was not influenced by VLCD.
Conclusion: Our results suggest that decreased mRNA expression of IGF-1 in SCAT and increased expression of IGFBP-3 in peripheral monocytes may induce local metabolic disturbances in adipose tissue contributing to development of insulin resistance and type 2 diabetes mellitus.
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Visceral fat reduction is associated with increased IL-10 levels in obese subjects that underwent caloric restriction

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Background and aims: Obese subjects are characterized by a low grade inflammatory state that may contribute in predisposing them to develop atherosclerosis. An excess of adipose tissue, particularly in intra-abdominal depots, is thought to play a role in the pathophysiology of the metabolic syndrome, closely linked to insulin resistance, and to increase the risk of cardiovascular disease. Although circulating levels of proinflammatory cytokines as well as other inflammation markers have been shown to be elevated in human obesity, little is known about the role of anti-inflammatory cytokines in this setting. IL-10 is a major inhibitor of cytokines synthesis; it suppresses macrophage function and inhibits the production of proinflammatory cytokines. Recent studies have shown an increase in IL-10 levels after caloric restriction. However, scant data exist about the effects on plasma IL-10 levels of the loss of visceral and/or subcutaneous fat tissue (VF and SF respectively). Aim of the present study was to verify whether changes in insulin sensitivity and in plasma levels of IL-10 together with several adipokines such as TNFα, IL-6 and Leptin specifically correlate with changes in VF or SF.
Materials and methods: We measured VF and SF by Magnetic Resonance (MRI), plasma levels of glucose, insulin, IL-10, TNFα, IL-6 and Leptin before and after a caloric restriction induced weight loss of at least 5% of the original body weight, in 14 (4 men, 10 women) obese subjects (BMI 34.4±6.5 Kg/mq).
Results: As we expected, weight loss improved insulin sensitivity (Quicki index= 0.35±0.03 vs 0.37±0.04, p<0.05), increased IL-10 levels (3.41±1.98 pg/ml vs 4.63±1,03pg/ml, p<0.05) and reduced TNFα , IL-6 e leptin levels (2.52±1.32 pg/ml vs 1.60±1.52 pg/ml; 2.32±0.42 pg/ml vs 1.64±0.64 pg/ml; 56.1±30.2 ng/ml vs 37.2±29.3 ng/ml respectively, p<0.05 for all). Moreover we observed a significant correlation between the amount of VF loss and the improvement in insulin sensitivity (r=0.44, p<0.05) and between the percent reduction in VF and the percent reduction in both TNFα (r=0.56, p<0.05) and IL6 (r=0.19, P<0.05) plasma levels. On the contrary, no correlation was observed between the investigated parameters and either the amount or the percentage of SF lost. Furthermore, in a multiple regression analysis, the amount of VF loss independently correlated with the increase in IL-10 levels.
Conclusion: These data suggest that the reduction in visceral but not in subcutaneous adipose tissue is associated with an improvement in the inflammatory pattern characterizing obesity and, specifically, that a loss in VF is associated with increased plasma levels of the anti-inflammatory adipokine IL-10.

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Weight loss in women is followed by a significant decrease of 11β hydroxysteroid dehydrogenase 1 in subcutaneous adipose tissue

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11ß hydroxysteroid dehydrogenase (11βHSD1) catalyzes the conversion of the inactive glucocorticoid compund cortisone into active cortisol, in a tissue-specific manner. This enzyme may have a key role in the development of central obesity and obesity-related complications. In humans, positive associations between 11βHSD1 in subcutaneous (sc) adipose tissue and different features of metabolic syndrome, including body mass index (BMI), blood pressure, and insulin sensitivity, have been reported. Furthermore, we have recently shown a positive correlation between 11βHSD1 and phosphoenolpyruvate carboxykinase C (PEPCK) - a key enzyme in gluconeogenesis. This supports a role for glucocorticoids in fatty acid (FA) metabolism in obesity, leading to increased rate of FA re-esterification in adipose tissue. In this study we evaluated peripheral glucocorticoid metabolism in the subcutaneous adipose depot before and after stabilized weight loss and explored a putative link between 11βHSD1 and fatty acid recycling in women. Twenty-seven obese women underwent gastric bypass surgery with collection of sc fat biopsies and blood before and two years after surgery. Computed tomography (CT) was used to estimate regional fat distribution and the amount of liver fat. Adipose tissue expression of 11βHSD1, leptin, adiponectin, PEPCK, sterol regulatory element binding protein (SREBP), fatty acid synthase (FAS), perilipin and hormone sensitive lipase (HSL) expression levels were analyzed with real-time PCR; serum leptin and adiponectin levels with ELISA. Two years after bypass surgery, there was a 69% mean decrease in BMI associated with a 2-fold reduction in the sc depot and 4-fold decrease in the intraabdominal depot. Liver attenuation decreased, reflecting decreased liver fat. 11βHSD1 gene expression decreased 4-fold after weight loss. Leptin and adiponectin expression decreased significantly, with concomitantly significantly decreased circulating leptin and increased adiponectin levels. There were no differences in the expression of genes involved in gluconeogenesis (PEPCK), lipolysis (perilipin, HSL), and lipogenesis (FAS, SREBP) after weight loss. Before surgery, sc 11βHSD1 gene expression correlated only to waist circumference, whereas after the significant weight reduction, 11βHSD1 mRNA levels correlated with multiple measures of central fat accumulation, ie. BMI, L4 total adipose tissue area and the intraabdominal fat area (p<0.01, p<0.05 and p<0.001; respectively). A significant correlation between 11βHSD1 and PEPCK observed before surgery was lost after weight loss (r=0.57, *P*<0.05 vs r=-0.02) respectively). Weight loss links to metabolically favorable changes in 11βHSD1 expression in fat and circulating levels of leptin and adiponectin. The reduction of 11βHSD1 in fat after weight loss suggests that up-regulation of this enzyme is a consequence, rather than a cause, of obesity. No significant differences in gene expression of the key enzymes involved in lipid metabolism and lack of associations to anthropometric measurements suggest that weight loss *per se* does not directly affect transcription of these genes in sc adipose tissue.
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11β- HSD1 in subcutaneous adipose tissue of SGA adults is dysregulated but not associated with metabolic disorders

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Background and aims: The mechanisms relating being born small for gestational age (SGA) and the later risk of metabolic disorders are not yet fully understood. Adipose 11β-HSD1 activity and expression has been positively associated with metabolic syndrome in animals and humans. In humans, no *in vivo* studies have explored 11β-HSD1 activity and gene expression in subcutaneous adipose tissue of SGA subjects.
Materials and methods: 39 subjects SGA, birth weight< 10th percentile, were matched on gender and age with 36 subjects born Appropriate for Gestational age (AGA - 25th <birth weight<75th percentile); the two groups were stratified according to body fat content into "low fat mass" (20 SGA and 18 AGA) and "high fat mass" (19 SGA and 18 AGA) subjects. Basal and stimulated activities of the 11β-HSD1 enzyme were assessed in the effluent of microdialysis performed in the abdominal subcutaneous wall *in vivo*. mRNA expression was measured by real-time quantitative PCR in subcutaneous adipose tissue.

Results:

	"low fat mass" AGA N= 18	"high fat mass"AGA N= 18	"low fat mass" SGA N= 20	"high fat mass" SGA N= 19	P low/high fat mass	P SGA/ AGA
Gender (M/F)	8/10	7/11	10/10	8/11		
Age (yr)	29.6 (26.1;33.3)	31.6 (26.7;32.9)	30.1 (24.7; 34.2)	32.6 (28.3 ; 35.0)		
Body fat (%)	21.8 (15; 28)	35.9 (29.5; 42)	22 (15; 27)	39 (28.7; 41)		
M clamp (mg/min/kg lean body mass)	12.5 (10.8; 14.8)	6.4 (4.8 ; 8.3)	10.5 (8.6 ; 15.0)	5.5 (4.1 ; 8.2)	*0.001*	0.68
Microdialysis						
Basal 11β-HSD1 activity (cortisol/ Cortisone)	1.7 (1.3; 1.9)	2.2 (0.9; 3.5)	1.9 (1.5 ; 2.5)	2.3 (1.5 ; 2.9)	0.83	0.69
Activation of 11β-HSD1 at nadir (%)	12.5 (5.7; 45.9)	18.8 (7.9; 95.7)	8.3 (5.3; 3.8)	7.3 (5.2; 15.1)	0.06	0.004
Fat Biopsy	N= 18	N=14	N=16	N=16		
Expression of 11β-HSD1 (arbitrary unit)	119 (90; 147)	249 (147; 407)	193 (112; 262)	341 (198; 421)	0.0001	0.18
Adipocyte diameter (μm)	84.5 (77.3; 89.8)	98.4 (90.3;110.3)	76.2 (56.4; 79.0)	99.3 (87.3;103.7)	<0.0001	0.003

Conclusion: The *in vivo* stimulated 11β-HSD1 activity was decreased in subjects born SGA as compared to adults born AGA. 11β-HSD1 gene expression was associated with body fat but not with birth weight. We also found an independent effect of both birth weight and body fat on adipocyte diameter. Moreover, expression and activation of 11β-HSD1 were strongly associated with the adipocyte diameter in SGA group, suggesting a "protective" role of the decreased size of adipocytes on the development of metabolic complications. It is therefore unlikely that local glucocorticoid metabolism in subcutaneous fat plays a major role in the development of the metabolic complications associated with being born SGA.

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Labisia pumila var alata extract down-regulates 11-beta hydroxysteroid dehydrogenase type-1 and corticosterone levels in overweight ovariectomized rats

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Background and aims: The enzyme 11-beta hydroxysteroid dehydrogenase type-1 (Hsd11b1) is highly expressed in key metabolic tissues including adipose and liver. In rats, it converts inactive 11-dehydrocorticosterone to active corticosterone (CORT). Activation of Hsd11b1 and glucocorticoid receptor could result in the production of excess tissue glucocorticoids which affects glucose homeostasis, insulin action and adiposity, all of which are associated with the development of type-2 diabetes and visceral obesity. Ovariectomy (OVX) rats have increased body weight and decreased insulin sensitivity in relation to estrogen deficiency. Our microarray analysis of liver of OVX rats has shown increased expression of Hsd11b1. Therefore, we examined Hsd11b1 expression and CORT levels on OVX rats after treatment with Labisia pumila var alata (LP), a Malaysian plant with phytoestrogen effects.
Materials and methods: Thirty-six Sprague-Dawley rats were ovariectomized (OVX) at 6-weeks of age and one group (N=7) undergone sham operation (SHAM). After two weeks, the rats were treated with oral gavage of LPva10, LPva20 and LPva50 extract (10, 20 and 50 mg/kg/day respectively), estrogen replacement (ERT) (0.625 mg/kg/day) for 30 days (n=7) or as controls (SHAM and OVX). Microarray analysis was done with liver tissue, followed by real-time RT-PCR of liver and adipose tissues. CORT levels in plasma were analyzed using ELISA and protein expressions were detected by Western blotting.
Results: OVX rats gained more body weight than SHAM rats (74.5 ± 3.7 g vs. 56.9 ± 3.6 g, $p < 0.05$). Treatment of OVX with LP50 or ERT significantly reduced the weight gain by 16.8% and 25.5%, respectively ($p < 0.05$ for both). CORT levels in OVX group increased significantly (135 ± 25 ng/ml, $p<0.05$) in comparison to SHAM (53 ± 22 ng/ml, $p<0.05$). The levels decreased in all LP10, LP20 and LP50 (85 ± 19 ng/ml, 95 ± 14 ng/ml and 92 ± 25 ng/ml respectively, $p < 0.05$ for all) and ERT (88 ± 16 ng/ml, $p < 0.05$). In adipose tissues, the Hsd11b1 mRNA level of OVX group increased by 55 % ($p<0.05$) in comparison to Sham, normalized in LPva10, LPva20 and LPva50 and but not significantly decreased in ERT treated rats. The Hsd11b1 mRNA levels in liver of OVX was increased by 75 % ($p < 0.05$) when compared to Sham and restored to normal level when given LPva extracts and ERT. Protein levels of Hsd11b1 were down-regulated in both adipose tissues and liver of LPva-treated rats, in comparison to Ovx rats (significant difference in all LPva groups and ERT, $p<0.05$).
Conclusion: OVX had increased body weight, increased adipose and liver expression of Hsd11b1 and elevated CORT levels. Treatment with LPva extracts, similar to ERT, normalized Hsd11b1 mRNA and protein levels in OVX rats, in parallel with decreased CORT levels. Based on our results, we hypothesize that anti-obesity effects of LPva are attributed, at least in part, to the inhibition of Hsd11b1 expressions in adipose tissue and liver. These changes suggest the use of LPva for a postmenopausal treatment and possibly, in other conditions related to metabolic syndrome.

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Transgenerational non-genomic inheritance of glucose intolerance by neonatal overfeeding in mice

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Background and aims: Epidemiologic evidence suggests that sub-optimal nutrition during foetal and/or postnatal development influences diabetes risk later in life. In addition, such environmentally-induced phenotypes may manifest in subsequent generations, even when the environmental triggers are not present anymore (transgenerational effects). We have previously generated a mouse model of neonatal over-nutrition (ON-F0) by culling offspring to 4 pups per dam during lactation. Neonatal overfeeding led to rapid early weight gain and later development of metabolic syndrome in adult mice, by age 6 months: i.e. obesity, dislipidemia, hyperglycaemia, hyperinsulinemia, insulin resistance and glucose intolerance.
Here we aimed to explore whether neonatal over-nutrition may influence metabolism of successive generations, F1 and F2.
Materials and methods: Control and ON males from the F0 generation were mated with non-sibling control females to generate the first generation-offspring, F1. At birth, all litters are adjusted to 8 pups per dam. Thus, contrary to the parental generation, ON-F1 pups are not neonatally overfed as com-

pared to their matched controls. We next repeated the breeding protocol, by using C-F1 and ON-F1 males, to obtain the second-generation offspring, F2. Likewise, all litters are equalized to 8 pups per dam to match normal nutrition during the neonatal period. Thus, an important consideration for the experimental design is that ON-F1 and ON-F2 male mice are not themselves overfed during lactation.
Results: We show that ON-F1 and ON-F2 male mice also develop several features of the metabolic syndrome, including fasting hyperinsulinemia (C= 0.22± 0.01 ng/ml; ON-F1= 0.44± 0.06**; ON-F2= 0.31± 0.05*; *p< 0.05, **p< 0.01), mild insulin resistance (HOMA-IR, C= 0.5± 0.04; ON-F1= 1.4± 0.21**; ON-F2= 0.7± 0.23, p= 0.1; **p< 0.01) and glucose intolerance by age 4 months. Impaired glucose tolerance in ON-F1 and ON-F2 mice appears be accounted for primarily by peripheral insulin resistance, since beta-cell function remains normal in these cohorts. Thus, here we show, for the first time, that neonatal overfeeding programs adult diabetes-related phenotypes not only to exposed individuals, but also to their offspring and grand-offspring. To note, transgenerational inheritance of insulin resistance occurs through the paternal lineage. Thus, transgenerational inheritance of the diabetic phenotypes must occur through, nutritionally-induced, modifications in cells of the germ line. It has been proposed that trans-paternal inheritance of such environmentally-acquired phenotypes might be mediated, in part, by epigenetic mechanisms.
Conclusion: In summary, nutritional challenges occurring during sensitive periods of development may have adverse metabolic consequences well beyond the lifespan of affected individuals and manifest in subsequent generations. Transgenerational progression of metabolic phenotypes through the male lineage supports a potential role for epigenetic mechanisms in mediating these effects.
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Paradoxical response in feeding in short-time fasted rAAV-leptin treated mice
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Background and aims: Leptin plays an impotant role in body weight regulation. Administration of leptin reduces food intake and body weight. However, we have shown that food intake did not decrease in rAAV-leptin treated (overexpression of leptin in the hypothalamus) wild type (wt) mice on a daily basis compared to control mice, though there was a decrease in body weight. To elucidate this discrepancy, we evaluated feeding behavior in short-term fasted rAAV-leptin treated wt mice.
Methods: rAAV-leptin and rAAV-GFP as a control was injected icv in wild type mice and daily (24 hours) food intake was measured. They were fasted for 3 hours and re-fed for 3 hours. Food intake was measured during the re-feeding period. Blood samples were collected before and after 3 hours fasting and blood glucose, plasma insulin, ghrelin and leptin levels were measured. Another set of mice (rAAV-leptin and rAAV-GFP treated) was fasted for 3 hours and glucose (1g/kg) was injected ip. The same blood samples were collected before glucose injection and 1 hour after glucose injection. Blood parameters same as above were measured in the same way.
Results: There was no change in total daily food intake in rAAV-leptin treated and control mice (control: 3.69Â±0.21, rAAV-leptin: 3.56Â±0.07 g). Food intake in rAAV-leptin treated mice during the re-feeding period was significantly increased compared to control mice (control: 0.19Â±0.04, rAAV-leptin: 0.38Â±0.05 g, p< 0.05). After fasting, blood glucose levels decreased (3.36Â±0.52 to 2.40Â±0.29 mmol/l, p< 0.01) in rAAV-leptin treated mice but not in control mice. Plasma ghrelin levels increased (1.32Â±0.32 to 3.92Â±0.75 ng/ml, p< 0.01) in rAAV-leptin treated mice but not in control mice. Plasma leptin levels decreased in control mice (5.45Â±0.79 to 3.81Â±0.45 ng/ml, p< 0.05) but stayed very low with no change in rAAV-leptin treated mice (0.36Â±0.07 to 0.29Â±0.04 ng/ml). Glucose injection decreased circulating ghrelin levels (11.10Â±1.21 to 2.42Â±0.28 ng/ml, p< 0.001) and increased leptin levels (0.42Â±0.12 to 0.77Â±0.15 ng/ml, p<0.05) in rAAV-leptin treated mice. No change was seen in the control group.
Conclusion: These results suggest that there was increased food intake when rAAV-leptin treated mice were fasted. Decrease in blood glucose or increase in circulating ghrelin in even short-term fasting periods may contribute to increased food intake in rAAV-leptin treated wt mice. This may explain, at least in part, the lack of difference in feeding behavior in rAAV-leptin treated wt mice and control mice. After glucose injection, reduction in circulating ghrelin and elevation in leptin levels may inhibit their increased food intake result in the normal daily food intake as if there is a feedback system.

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Reduced insulin gene dosage prevents diet-induced obesity
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Background and aims: Obesity has become a worldwide epidemic and can often lead to insulin resistance, hyperinsulinemia and type 2 diabetes, but the relationship amongst these phenomena remains enigmatic. Although insulin is a potent adipogenic hormone, it is thus far unclear whether hyperinsulinemia itself can be a causal factor in the pathogenesis of obesity and/or type 2 diabetes. Unlike humans, mice possess two non-allelic insulin genes. It has been previously established that mice lacking one of their two insulin genes are phenotypically normal. Furthermore, it is reported that only *Insulin 1* (*Ins1*) is exclusively expressed in the pancreatic beta cells whereas *Insulin 2* (*Ins2*) is also expressed in extra-pancreatic tissues where it can have non-endocrine functions. We therefore hypothesized that mice with only one active *Ins1* allele would be protected from diet-induced obesity and associated pathologies.
Materials and methods: To test hyperinsulinemia as an endocrine pathology, we used *Ins2* knockout mice. We compared mice with only a single copy of *Ins1* (Ins1+/-:Ins2-/-) with those with two copies of *Ins1* (Ins1+/+:Ins2-/-) with respect to the adverse effects of high-fat feeding. We studied body weight, glucose- and insulin- tolerance, insulin mRNA levels, islet insulin content, total body lipid content by NMR, epididymal fat pad weight, lipid accumulation in multiple tissues, and pancreatic beta cell mass by immunofluorescence microscopy.
Result: Ins1+/-:Ins2-/- mice were protected from adult-onset diet-induced weight gain compared with Ins1+/+:Ins2-/- controls ($P < 0.01$). High-fat fed mice with one or two active *Ins1* gene allele showed early elevated levels of circulating insulin when compared to chow fed mice of either of the genotypes. This was positively correlated with a slight increased overall body growth as per measured by tibial length at one year of age. However, one year old high-fat fed Ins1+/-:Ins2-/- mice had significantly reduced basal circulating insulin levels ($P < 0.01$), reduced insulin response to glucose stimulation, lower beta cell mass, lower whole-body fat ratio ($P < 0.05$), smaller epididymal fat pads ($P < 0.01$), smaller adipocytes, and no hepatic steatosis when compared with Ins1+/+:Ins2-/- mice on the same diet. Mice of either genotypes on the chow diet showed comparable phenotypes and were similar to that of observed in the high-fat fed Ins1+/-:Ins2-/- mice. Mice across all groups showed normal and comparable glucose tolerance and insulin sensitivity.
Conclusion: We have shown that prevention of diet-induced hyperinsulinemia through partial ablation of insulin gene can protect mice from obesity and its associated complications. Hyperinsulinemia may not just simply be an adaptive response to obesity-induced insulin resistance and may play a causal role in the pathogenesis of obesity and/or diabetes. Therapeutic interventions that reduce circulating insulin may be worth exploring in the context of obesity and pre-diabetes.
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PS 70 Adipocyte biology: new kids on the block

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Micro-ribonucleic acid expression profiling and expression quantitative trait loci analysis in human gluteal and abdominal adipose tissue

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Background and aims: Obesity is a large and growing public health problem associated with increased risks of type 2 diabetes, cardiovascular disease, hypertension and increased mortality. Adipose tissue distribution relates to morbidity with increased levels of abdominal adipose tissue relative gluteal adipose tissue associated with metabolic deregulation and related disease. Hence, characterization of the molecular phenotypes in these two adipose tissues is an essential starting point when attempting to understand molecular mechanisms associated with obesity and related disease. Here we characterise micro-RNA (miRNA) expression and how it contributes to the molecular phenotype in gluteal and abdominal adipose deposits, which are known to affect risk of obesity-related disorders.
Materials and methods: The expression of 1131 miRNAs was profiled in these two fat depots in 70 human subjects using the Illumina DASL miRNA beadarray. The study includes male and female subjects diagnosed with metabolic-syndrome as well as healthy controls. Here we focus on investigating tissue-specific miRNA expression. All subjects were genotyped using the Illumina HumanHap317 Beadchip, enabling assessment of whether there are individual genetic variants driving miRNA expression levels, by miRNA expression quantitative trait loci (eQTL) analysis. Tissue-differential expression was analysed using a linear mixed effects model with miRNA expression as response; tissue type, batch, gender, metabolic-syndrome status and age included as fixed effects; and a subject identifier included as random effect. Significance of the tissue effect was assessed by a permutation test of the likelihood-ratio statistic. A similar model and test was used for eQTL analysis, with the genotype included as an additive fixed effect. eQTL models were fitted separately for each tissue type.
Results: We found that 154 miRNAs were differentially expressed between gluteal and abdominal fat tissue (FDR corrected (Benjamini and Hochberg's method) p-value < 0.05). These miRNAs include hsa-miR-211 (p-value=0.006), hsa-miR-27b (p-value=0.006), hsa-miR-27a (p-value=0.011), hsa-miR-34a (p-value=0.020) and hsa-miR-143 (p-value=0.020), which have previously been reported to be associated with adipose tissue development, obesity and metabolic disorders. We detected 10 miRNA-eQTL candidates in gluteal adipose tissue and 23 miRNA-eQTL candidates in abdominal adipose tissue (nominal p-values < 0.002). Currently we are undertaking a confirmation study in 40 additional subjects, with the objective of replicating the current findings.
Conclusion: The results indicate a substantial difference in miRNA expression patterns between gluteal and abdominal adipose tissues, which may indicate an important role of miRNAs in contributing to the molecular phenotype of these two tissue types. Presence of miRNA-eQTLs indicate a direct genetic influence on the expression of specific miRNAs, and consequently also indirectly on the general molecular phenotype of gluteal and abdominal adipose tissue.
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Human adipose tissue - a novel source of eotaxin-3

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Background and aims: Obesity and overweight are major risk factors for chronic diseases, including type 2 diabetes, cardiovascular disease, hypertension and stroke, as well as certain forms of cancer. Obesity is characterized by a chronic, systemic low-grade state of inflammation. Adipose tissue secretes a multitude of factors, e.g. adipokines and cytokines that are involved in the body's homeostasis and metabolism. A causal relationship of obesity associated inflammation with metabolic disorders is indicated. We aimed at identifying cytokines released from human adipocytes and identified eotaxin-3 (CCL26). Eotaxin-3 is a member of the CC chemokine family, known to be expressed in several tissues, e.g. heart and lung, and is an important effector chemokine in allergic conditions. Eotaxin-3 acts mainly via the CCR3 receptor and is a potent chemoattractant for eosinophils. Additionally, evidence exists that eotaxin-3 acts as an antagonist on chemokine receptors CCR1, CCR2 and CCR5, suggesting a modulatory function of eotaxin-3 in inflammation. No data, however, exists so far on the expression in and/or secretion of eotaxin-3 from adipose tissue.
Materials and methods: Mature adipocytes and preadipocytes were isolated from white human adipose tissue obtained from healthy women undergoing surgical mammary reduction or liposuction. Preadipocytes and *in vitro* differentiated adipocytes were stimulated with IL-4, TNFα, and/or IFNγ. Visceral and subcutaneous fat tissue was obtained from patients undergoing abdominal surgery. Eotaxin-3 gene expression was determined with quantitative PCR. Eotaxin-3 secretion was measured with a specific RIA.
Results: In the present study, eotaxin-3 was expressed in most samples of human adipocytes and secreted constitutively. Intra-individual comparism of eotaxin-3 gene expression from subcutaneous and visceral fat depots showed higher eotaxin-3 levels in subcutaneous fat tissue. The eotaxin-3 expression in subcutaneous fat tissue is correlated to donor BMI. Furthermore, its expression was higher in adipose tissue than in isolated adipocytes. Preadipocytes and *in vitro* differentiated adipocytes expressed and secreted eotaxin-3 at low levels; its expression and secretion, however, was strongly induced by stimulation with IL-4, via STAT6 pathway, but not TNFα or IFNγ. Co-stimulation of preadipocytes with IL-4 and IFNγ decreased IL-4 induced eotaxin-3 expression; prestimulation with IFNγ decreased IL-4 induced eotaxin-3 expression in a dose-dependent manner.
Conclusion: We could identify human adipocytes as a novel source of eotaxin-3. Eotaxin-3 expression in subcutaneous fat tissue was positively correlated with BMI. Its expression is inducible by IL-4, an anti-inflammatory cytokine produced e.g. by adipose tissue. IFNγ inhibits IL-4 induced eotaxin-3 expression. A possible role of eotaxin-3 in obesity-associated disorders needs to be elucidated.

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Human adipocytes express P2X7 receptors able to modulate some inflammatory responses in subjects with metabolic syndrome

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Backgound and aims: No information is available on the presence of P2X7 receptor in human adipocytes and their potential involvement in the chronic inflammation associated with metabolic syndrome (MS). Adipocytes were isolated from samples of visceral (VAT) and subcutaneous (SAT) adipose tissue of 40 patients with MS (defined as by ATP-III criteria) and 20 controls (CLT), recruited among patients undergoing laparoscopic cholecistectomy.
Materials and methods: We measured adipocyte gene expression of TNFα,IL-6 and PAI-1 (by realtime-PCR) and their plasma concentrations (ELISA), as well as gene and protein expression of P2X7 (using realtime-PCR, Western blot and immunocytochemistry). In a subgroup of 15 MS and 10 CLT we also evaluated P2X7 functional activity by measuring the effect of benzoyl-benzoyl-ATP (BzATP, a P2X7 specific agonist) and KN62 (a selective P2X7 blocker) on intracellular calcium fluxes (([Ca2+]I, by fluorimetry) and adipocytoke release (by ELISA).
Results: In VAT, TNFα, IL-6 and PAI-1 were more espressed in MS than in CLT (T/R ratio: 3.29±1.47 *vs* 1.79±1.19; 8.99±4.24 *vs* 5.65±4.03; 6.06±2.32 *vs* 2.91±0.34, p =0.005-0.0001). These differences were confirmed in SAT for IL-6 (3.56±1.56 *vs* 1.98±1.49, p=0.0004) and PAI-1 (3.87±1.87 *vs* 2.25±1.16, p=0.008), but not for TNFα (1.49±0.83 *vs* 1.24±0.68, p=ns). Plasma IL-6 and PAI-1 levels were higher in MS (IL-6: 2.81±1.55 *vs* 4.32±2.67 pg/ml, p=0.002; PAI-1: 32.08±11.21 *vs* 42.26±11.6 ng/ml, p=0.002). TNFα levels were higher in MS (3.22±1.26 *vs* 2.51±0.88 pg/ml, p<0.05). P2X7 mRNA, found both in VAT and SAT, was more abundant in MS than in CLT (T/R ratio: 2.13±0.68 *vs* 1.56±0.49 in VAT, p=0.0013 and 1.76±0.54 *vs* 1.46±0.41 in SAT, p=0.03). Protein expression confirmed this observation, with the typical "ring-like" arrangement of P2X7 receptor at the plasma membrane. BzATP 0.5 mM raised [Ca2+]i in VAT and SAT, without differences between MS and CLT (VAT:

+128 *vs* +98%, p=ns; SAT: +107 *vs* +110%, p=ns). In both MS and CLT cells BzATP induced IL-6 and TNFα release, partially inhibited by KN62 (VAT: IL-6 from 141±33 to 308±28 and 233±33 with KN62 in CLT; from163±27 to 318±64 and 254±59 pg/ml/mg tissue with KN62 in MS, p<0.0001; TNFα from 2.3±0.7 to 3.7±0.8 and 3.1±0.9 with KN62 in CLT; from 2.3±0.6 to 4.0±0.8 and 3.2±0.9 pg/ml/mg tissue with KN62 in MS, p<0.0001). BzATP did not induce any change in PAI-1 release, either in MS or CLT.
Conclusion: Human adipocytes express functionally active P2X7 receptors, which modulate the release of some inflammatory cytokines. Adipocytes from MS patients show an enhanced P2X7 receptor expression, which might contribute to the subclinical inflammatory status characterising these patients.

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OCT-1 expression in adipocytes could contribute to increased metformin action in obese subjects
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Background and aims: Metformin is an insulin-sensitizer widely used to treat type 2 diabetes mellitus. The metabolic effects of metformin on human adipocytes have not been well studied. Organic cationic transporters (OCT-1 and OCT-2) have been described to mediate metformin effects. We investigated the expression of *OCT-1* and *OCT-2* in human adipose tissue and during adipogenesis and evaluated their possible role in metformin action on human adipocytes.
Materials and methods: *OCT-1* and *OCT-2* gene expressions were analyzed in 118 visceral and subcutaneous adipose tissue, in stromo-vascular cells (SVCs) and mature adipocytes obtained from adipose tissue and during human pre-adipocytes differentiation. To test the functionality of OCT in response to metformin, co-treatments with cimetidine (OCT blocker, 0.5 and 5 mM) and metformin (5 mM) were made on human pre-adipocytes. The pre-adipocyte differentiation was monitored measuring adipogenic (*FASN, ACC1*, *PPARg* and *Adipoq*) and inflammatory (*IL-6* and *MCP-1*) gene expressions, the formation of lipid droplets and AMPK activity.
Results: *OCT-1* (but not *OCT-2*) gene expression was detected in subcutaneous and visceral adipose tissue. In both fat depots, *OCT-1* gene expression and protein was associated significantly with the obesity phenotype. *OCT-1* gene expression was significantly higher in SVCs than mature adipocytes (1.8-fold increased, p=0.01) and increased during differentiation the process in parallel to adipogenic genes. Metformin (5mM) decreased significantly the differentiation of human pre-adipocytes, decreasing the expression of lipogenic genes, lipid droplets accumulation, increasing AMPK activation. Co-treatments with cimetidine restored adipogenesis. Furthermore, metformin decreased *IL-6* and *MCP-1* gene expression in comparison with differentiated adipocytes.
Conclusion: OCT-1 might mediate the action of metformin on human adipose tissue.
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The role of proliferin (PLF) in adipose tissue
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Background and aims: Last year, we reported that mature adipocytes have the ability to proliferate, which is enhanced with pioglitazone (Pio) treatment. We demonstrated that 5-Bromo-2'-Deoxyuridine (BrdU), a thymidine analog, incorporation into mature adipocytes was observed in BrdU-pretreated rats. Quantified results showed that amount of BrdU incorporation was equal in adipocytes and total stromal vascular fraction cells, and that BrdU incorporation was less active in subcutaneous adipocytes than visceral ones. Treatment with 0.005% Pio containing food for 2w increased BrdU incorporation predominantly in subcutaneous, but not visceral adipocytes. Moreover, we found that an increase in cell number and the cell cycle analyzed with flow cytometry were recognized in fully differentiated 3T3-L1 adipocytes. Treatment with PPARγ ligands, 10 μM Pio and 10 μM 15-deoxy-Δ12,14-prostaglandin J2 increased adipocytes proliferation. In this study, we screened genes regulated by Pio, and we took note of PLF as a growth factor in adipocytes. PLF, also termed mitogen-regulated proteins, is a member of the prolactin/GH family is involved in angiogenesis of placenta. PLF, secreted as a paracrine agent, binds to the insulin-like growth factor II (IGF-II)/cation-independent mannose 6-phosphate (M6P) receptor which can couple with Gi protein. We clarify the role of PLF in adipocytes replication.
Materials and methods: Effects of PLF knockdown with siRNA and treatment with anti-PLF antibody (PLF-Ab) on 3T3-L1 adipocytes were examined. Expression of PLF in adipocytes isolated from rats during treatment with or without Pio was measured. In addition, effect of PLF-Ab administration on adipocytes growth was evaluated.
Results: Treatment with Pio increased PLF mRNA levels and protein levels in cell lysate and medium in 3T3-L1 adipocytes. Both PLF knockdown and an addition of PLF-Ab reduced adipocytes proliferation evaluated by cell number and BrdU incorporation. Treatment with IGF-II enhanced cell proliferation, whereas pertussis toxin suppressed its proliferation. These results indicated that PPARγ-induced PLF positively regulates cell replication in 3T3-L1 adipocytes. and that involvement of IGF-II/cation-independent M6P receptor was also suggested. PLF was more abundantly expressed in visceral adipocytes than subcutaneous ones, while treatment with Pio increased subcutaneous adipocytes, but not visceral ones, which closely correlated to the extent of BrdU incorporation in each adipose tissue. PLF-Ab injection resulted in decreased BrdU incorporation in adipocytes. These results strongly supported the notion that PLF is actually involved in mature adipocyte replication in vivo.
Conclusion: PLF was suggested to be involved in cell replication as a PPARγ-induced growth factor in mature adipocytes.

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Mammalian Ste20 kinase stimulates adipogenesis by activation of PPARγ
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Background and aims: Recent studies have shown that mammalian ste20 kinase (MST) signaling pathway plays an important role in the regulation of apoptosis and cell cycle control and is thus emerging as a novel tumor suppressor pathway. MST pathway, originally identified as hippo pathway in Drosophila, involves several participating proteins including a scaffolding protein Salvador (SAV1). The idea of MST signaling pathway functioning in cell differentiation seems plausible but needs investigation. Based on our finding that MST2 interacts with PPARγ, a key regulator of adipogenesis, through SAV1, we sought to understand the novel role of the MST signaling pathway in adipocyte differentiation.
Materials and methods: Proteins of interest were overexpressed or knocked down by siRNA in cell cultures of HEK293 cells and 3T3-L1 adipocytes. We employed various protein analysis methods and Oil red staining to analyze the MST signaling pathway.
Results: We found that MST2 bound to PPARγ through SAV1 and stabilized PPARγ protein. Interaction was dependent on the N-terminal portion of SAV1 including WW domains and the N-terminal portion of PPARγ including PPXY motif. We could not find the evidence that PPARγ is a direct substrate of MST2. Coexpression of MST2 and SAV1 resulted in a profound induction of PPARγ activity even in the absence of stimulation by an agonist rosiglitazone. During the differentiation period of 3T3-L1 cells, the protein expression of MST2 and SAV1 showed increases preceding that of PPARγ and the protein complex of endogenous SAV1 and PPARγ could be detected. Finally, adipocyte differentiation of 3T3-L1 cells was increased by overexpression of MST2 and SAV1 while it was decreased by knockdown of SAV1 using siRNA.
Conclusion: From the results, we propose that the activation of PPARγ by the MST signaling pathway may be a novel and important regulatory mechanism of adipocyte differentiation.

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Association of human obesity with altered adipose PTX3 gene expression and protein plasma levels

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Background and aims: PTX3 is a secreted acute-phase long pentraxin that is believed to mediate innate immunity and inflammation. PTX3 gene is mainly expressed in adipose tissue, and smooth and cardiac muscle in humans. Plasma PTX3 protein concentration has been negatively correlated with human obesity, and positively with atherosclerotic inflammation, and is a risk marker for myocardial infarction. The PTX3 gene expression is stimulated by TNFα and IL-1β in cultured human fibroblasts and endothelial cells. We examine the relationship between plasma PTX3 protein and adipocyte PTX3 gene expression levels with human obesity. We also aim to provide insight into the mechanisms of PTX3 alterations.
Materials and methods: Subjects: Two different cohorts were included in the study. For adipose tissue gene expression, 43 obese and 19 lean age- and gender-matched subjects were selected. A second cohort of 75 apparently healthy men (mean age 50.7 ± 11.2 years) was selected for the study of insulin sensitivity and insulin secretion using the minimal model approach. Methods: Paired subcutaneous (sc) and visceral (v) adipose tissue (AT) biopsies and isolated adipocytes. PTX3 gene expression was assessed by quantitative real time PCR. PTX3 protein plasma levels were measured by ELISA. Human preadipocyte SGBS cell line was differentiated *in vitro* to mature adipocytes and subjected to various stimuli.
Results: A negative correlation between plasma PTX3 protein levels, body weight (r=-0.32, p=0.016) and waist-hip ratio (r=-0.37, p=0.006) was shown. We also found a negative correlation between plasma PTX3 protein and total triglyceride levels (r=-0.33, p=0.004), and insulin secretion after intravenous glucose administration (acute insulin response to glucose) (r=-0.34, p=0.006) and oral glucose administration (measured as serum insulin at 30 minutes of the oral glucose tolerance test) (r=-0.25, p=0.04). Total PTX3 gene expression was similar in vAT and scAT, regardless of obesity. Expression in vAT was significantly lower in non-obese (BMI ≤ 25 Kg/m^2) than in obese (BMI > 25 Kg/m^2) subjects (p=0.039). The PTX3 gene expression was higher in adipocytes isolated from vAT than from scAT (p=0,034). Likewise, in vAT, the PTX3 gene was more strongly expressed in the isolated adipocytes than in the stromovascular fraction (p=0,028). In cultured SGBS adipocytes, PTX3 gene expression was enhanced by TNFα and IL-1β, whereas IL-6, insulin, hypoxia, antimycin A and H_2O_2 had no significant effect.
Conclusion: Plasma PTX3 protein levels correlate negatively with obesity markers and insulin secretion in human subjects. In contrast, the PTX3 gene expression is upregulated in vAT depots of obese subjects and it is enhanced in cultured human adipocytes by proinflammatory cytokines. These data indicate that PTX3 may have a role in the inflammatory process that drives obesity.
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Ask1 is regulated in intra-abdominal adipocytes and human fat through combinatorial phosphorylation input and by stress-specific transcriptional regulation

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Background and aims: We have recently demonstrated that omental fat in human obesity is associated with a MAP kinase stress signaling pathway involving the MAP3K5 Ask1 in adipocytes. Moreover, Ask1 exhibited both increased levels of the activating phosphorylation site Thr845, and increased protein and mRNA expression, the latter demonstrated as an independent predictor of whole-body insulin sensitivity. Here we aimed at unraveling the molecular basis for the regulation of Ask1 in intra-abdominal adipocytes.
Materials and methods: We utilized novel differentiated depot-specific pre-adipocyte cell lines, which we have demonstrated to retain various depot-specific characteristics to study phosphorylation- and expression mediated regulation of Ask1 by Western blot and qRT-PCR analyses, respectively. Findings were also determined in human omental and subcutaneous (SC) adipose tissue.
Results: Intra-abdominal (IA) adipocytes exhibited larger increase in Thr845 phosphorylation than subcutaneous (SC) adipocytes in response to TNFα. In contrast, TNF-induced phosphorylation on the inhibitory Ser83 site was lower in IA adipocytes and peaked later than the activatory site. Like p-Ser83, Thr966 phosphorylation also peaked later than Thr845 in both cell lines. Ask1 mRNA levels increased approximately 2-fold in response to TNFα (at 4 and 24h), FasL and oxidative stress (at 24h), but no activation was observed with the ER stress inducer tunicamycin. Intriguingly, in human omental compared to SC fat despite elevated p-Thr845, different levels of the inhibitory phosphorylation sites could be observed, suggesting that activity is influenced by a combinatorial phosphorylation input. With regards to gene expression, we assessed members of the E2F family of transcription factors, since they have been implicated in the regulation of Ask1 expression in-vitro. Human omental fat displayed increased mRNA and protein expression of members of the transcription factors members E2F1 and E2F4 in omental versus SC fat, and in obesity compared to lean persons.
Conclusion: Ask1 in intra-abdominal adipocytes may be regulated in a complex manner: i. by a combinatorial input of Ask1 kinases acting on activatory and inhibitory phosphorylation sites. ii. by changes in gene regulation, potentially mediated by changes in E2F family of transcription factors in response to inflammatory and oxidative stress.
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TNF-like inducer of apoptosis prevents TNF-alpha-induced insulin resistance in human visceral adipocytes

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Background and aims: Insulin resistance is an important contributor to the pathogenesis of type 2 diabetes (T2D) and obesity is a risk factor for its development. In the obese state an altered secretion pattern, with increase in pro-inflammatory and decrease in anti-inflammatory factors is found. In fact, obesity is considered a low-grade inflammatory state. Several mediators released from adipocytes and macrophages, including TNF family members, have been suggested to impair insulin action in peripheral tissues. Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a relatively recently identified pro-inflammatory cytokine that regulates multiple cellular responses. Tweak could be involved in the pathogenesis of chronic inflammatory diseases but its physiological role in adipose tissue is still not known. The objective of the present study was to dissect the differential effects of Tweak vs. TNF-α on human visceral adipocytes.
Materials and methods: We analyzed the impact of TNF-α and Tweak on glucose uptake and insulin action in a human visceral adipocytic cell line with high capacity to differentiate.
Results: TNF-α and Tweak activates different intracellular signaling pathways in human visceral adipocytes. In this regard, TNF-α induces insulin resistance by JNK1/2-dependent mechanism, impairing insulin-stimulated glucose uptake and insulin signaling at the insulin receptor substrate (IRS)-1/Akt level. By contrast, Tweak does not induce JNK1/2 activation and in consequence insulin sensitivity on glucose uptake is not affected. Moreover, pre-treatment with Tweak prevented TNF-α-dependent JNK1/2 activation, restoring insulin signaling and insulin-induced glucose uptake.
Conclusion: Tweak shows a protective role against TNF-α-induced insulin resistance in human visceral adipocytes. Our results suggest the balance of TNF family members on adipose tissue as a key factor in the pathogenesis of obesity-associated metabolic disorders.
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Adipose reduction in the activity of the repressor ICER is responsible for insulin resistance elicited by the transcriptional factors CREB in obesity

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Background and aims: Sustained adipose activation of the transcriptional activators cAMP response binding proteins (CREB) in obesity leads to impaired expression of the glucose transporter GLUT4 and adiponectin (adipoq) in mice model of obesity. Diminution of GLUT4 and adipoq caused by CREB is indirect and relies on the increased repressive activity of the CREB target gene activating transcription factor 3 (ATF3). Specific inactivation of CREB in adipocytes decreases ATF3 production and improves whole-body insulin sensitivity of mice in the context of diet-induced obesity. Thus, elevation of CREB activity is a key mechanism responsible for adipocyte dysfunction and systemic insulin resistance. The inducible cAMP early repressor (ICER) is a negative regulator of the CREB activity. In fact, ICER antagonizes the CREB factor by competing for the regulation of similar target genes. The goal of the study was to investigate whether loss of ICER expression in adipocytes could be responsible for increased CREB activity in obesity.
Materials and methods: Mice C57bl6 were fed with a high fat diet (HFD) for 12 weeks to increase body weight and generate insulin resistance. Biopsies of visceral adipose tissues (VAT) were prepared from human lean (BMI=24±0.5 Kg/m2) or obese subjects (BMI>35 Kg/m2). Total RNA and protein were prepared from white adipose tissues (WAT) of chow- or HFD-fed mice and VAT of lean and obese subjects. Activities of CREBs and ICER were monitored by electromobility shift assays (EMSA). The role of ICER on CREB activity was confirmed in 3T3-L1 adipocytes cells. Briefly after differentiation, the cells were electroporated with the plasmid coding for ICER cDNA. Gene expression was quantified by quantitative real-time PCR and western Blotting experiments.
Results: The expression of ICER is reduced in WAT of HFD-induced obese mice when compared to chow mice as measured by real-time PCR and EMSA. Similar result was found in human tissues. Reduction in ICER expression was associated with increased ATF3 expression and decreased adipoq and GLUT4 contents. Diminution in ICER levels was observed in adipocytes fraction whereas its expression was unchanged in stroma vascular fraction of WAT. Overexpression of ICER in 3T3-L1 adipocytes silenced the expression of ATF3, confirming the regulation of the factor by ICER. The expression of ICER is regulated by histone deacetylases activity (HDAC). Inhibition of HDACs in 3T3-L1 adipocytes cells using trichostatin inhibited the production of ICER. The whole activity of HDAC was reduced in WAT and VAT of obese mice and human obese subjects.
Conclusion: Impaired adipose expression of ICER is responsible of increased CREB activity in adipocytes in obesity. This mechanism relies on reduction of the HDAC activity.

PS 71 Adipose tissue inflammation

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IL-1β is a putative mediator in disturbed adipocyte-hepatocyte crosstalk that is induced by adipose inflammation

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Background and aims: Although the role of IL-1β in (auto)inflammatory cascades is well established, its involvement as an endocrine mediator of adipose tissue-liver crosstalk in obesity is still debated. Here we hypothesized that secreted IL-1β by adipose tissue plays a role in mediating hepatic insulin resistance in response to adipose inflammation.
Materials and methods: We utilized primary rat hepatocytes or human and rat hepatoma cell lines, and tested direct modulation of proximal and distal insulin signalling by IL-1β. In addition, we used conditioned medium from human omental fat explants. Furthermore, we measured systemic and portal IL-1β levels and mRNA expression in different fat depots in mice.
Results: The release of IL-1β from human omental fat explants ranged between 0.15 and 1.5 ng/gr tissue per 24h, and correlated with BMI (R^2=0.639, p<0.01). Human hepatoma cells (HepG2) incubated with omental fat conditioned medium exhibited impaired insulin-stimulated phosphorylation of IR, IRS, PKB and GSK3. This effect has been strongly attenuated (p<0.05) by co-incubation with human recombinant IL-1 receptor antagonist (IL-1Ra). Mice fed high fat diet for 8 weeks (i.e., before marked infiltration by macrophages occur) exhibited no detectable increase in IL-1β mRNA in the systemically-drained peripeipdidymal fat pad, but a 1.8-fold increase in IL-1β expression in portal-drained mesenteric fat. Consistently, whereas no increase in systemic circulating IL-1β levels was observed in these mice, portal blood levels of the cytokine were 2.4-fold elevated (p<0.05). The potential of IL-1β to induce insulin resistance was further demonstrated utilizing rat primary hepatocytes, and in the Fao hepatoma cells could be shown to engage impaired insulin-stimulated tyrosine phosphorylation of both IRS1 and IRS2.
Conclusion: While clear pathophysiological role for IL-1β as a mediator of hepatic insulin resistance in response to adipose inflammation still awaits appropriate in-vivo models, this study provides compelling in-vitro support for this notion. Reported beneficial effects of IL-1Ra or neutralizing anti-IL-1β Ab may therefore prove to be at least partly contributed by interference with a dysfunctional visceral fat-liver communication.
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The role of IL-1RI mediated T cell accumulation in adipose tissue - insights to the development of obesity-induced insulin resistance

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Background and aims: Immune cell infiltration into adipose tissue during high-fat feeding has recently been characterised with T cell recruitment evident prior to macrophage recruitment. Immune cell-derived cytokines are hypothesized to augment adipose tissue inflammation and insulin resistance. Cytotoxic T cells are effector T cells that have been implicated in adipose tissue macrophage differentiation, activation and migration. Helper T cells are a sub-group of T cells involved in the activation and direction of other immune cells. γδ T cell receptor (TCR) expressing cells represent a small subset of T cells that possess a distinct γδ TCR on their surface and play a key role in amplifying the immune response bridging the innate with the adaptive immune system. IL-1 plays a key role in activation of γδ TCR expressing cells and thus in this study we hypothesized that lack of IL-1 signalling may alter T cell subset recruitment into obese adipose tissue, in particular affecting γδ TCR expressing cell recruitment.
Materials and methods: C57BL/6 WT and IL-1RI$^{-/-}$ mice were fed a high-fat diet (HFD) (45 % palm oil) for 16 weeks. At weeks 0, 6, 12 and 16, glucose tolerance tests (GTT) were performed (1.5g/kg glucose, ip) and epididymal

adipose tissue (EAT) was harvested. Adipocytes and stromal vascular cells (SVC) were separated by collagenase treatment. SVC were labelled with antibodies for T cell markers CD3, CD4, CD8 and the γδ T cell receptor (TCR) and analysed by flow cytometry. $CD3^+CD4^-CD8^+$ cells are cytotoxic T cells while $CD3^+CD4^+CD8^-$ cells represent helper T cells. Cells expressing the γδ TCR were also determined. Results presented as percentage total SVC.
Results: IL-$1RI^{-/-}$ mice exhibited a more glucose tolerant phenotype at baseline and after 12 and 16 weeks on HFD. Development of obesity was associated with a steady rise in the number of cytotoxic T cells ($CD3^+CD4^-CD8^+$) in adipose tissue of WT mice after 6 (from 0.06±0.02% to 2.45±0.3%) and 12 (6.57±1.2%) weeks of high-fat feeding ($p<0.001$), with no difference between WT and IL-$1RI^{-/-}$ genotypes. The population of helper T cells ($CD3^+CD4^+CD8^-$) remained low throughout with significantly higher levels observed in WT mice at week 16 compared to IL-$1RI^{-/-}$ (5.8±0.7% vs 2.8±0.9%) ($p<0.001$). Interestingly the number of γδ TCR expressing cells recruited into adipose tissue during HFD was much higher (19.3±1.5% at week 12) than other T cell subsets. Further, there was significantly less in the IL-$1RI^{-/-}$ mice compared to WT after 12 (19.3±1.5% vs 15.3±1.0%) and 16 (13.4±0.7% vs 9.5±1.4%) weeks of high-fat feeding ($p<0.05$) consistent with an important role for IL-1 in activation of γδ TCR expressing cells.
Conclusion: In the present study we demonstrate that during obesity both cytotoxic T cells and γδ TCR expressing cells infiltrate adipose tissue and thus may play a crucial role in initiating the inflammatory response and recruiting adipose tissue macrophages. Further, IL-1 plays a key role in mediating infiltration of γδ TCR expressing cells into adipose tissue but does not affect recruitment of other T-cell subsets. Reduced recruitment of γδ TCR expressing cells into adipose tissue of IL-$1RI^{-/-}$ mice may in turn partially account for protection from HFD-induced insulin resistance.
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Human adipose tissue macrophage activation and impairment of adipocyte functions by osteopontin
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Background and aims: Osteopontin (OPN) is highly upregulated in adipose tissue in human and murine obesity and has been recently shown to be functionally involved in the pathogenesis of obesity-induced adipose tissue inflammation and associated insulin resistance in mice. OPN is a protein with multiple functions and acts as a chemokine and an inflammatory cytokine through a variety of different receptors (CD44, integrins). It is expressed in many cell types including adipose tissue macrophages (ATM). However, the target cells of OPN action in obese adipose tissue are still elusive. Here, we investigated expression of OPN receptors and the impact of OPN on ATM, adipocytes and other cells of human adipose tissue.
Materials and methods: Receptor expression was assessed by immunostaining of human omental adipose tissue sections and mRNA expression in fractionated subcutaneous adipose tissue. Human in vitro differentiated macrophages and primary adipose tissue macrophages isolated by flow-cytometry were stimulated with OPN. Human adipocytes differentiated from primary preadipocytes were pretreated or not with OPN prior to insulin stimulation.
Results: We found broad expression of OPN receptors in different adipose tissue cell types including adipocytes. OPN stimulated phosphorylation of Akt and MAP kinases, degradation of IκB-α, as well as secretion of Mcp-1, TNFα, and IL-10 in model macrophages and isolated human ATM. Moreover, OPN impaired differentiation and function of primary adipocytes as determined by PPARγ and adiponectin gene expression and insulin-stimulated glucose uptake.
Conclusion: OPN activates adipose tissue macrophages and interferes with adipocyte function thereby underlining the potential use of OPN as a therapeutic target for obesity-induced complications.
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High-fat diet induced insulin resistance triggers infiltration of dendritic cells into adipose tissue and primes the inflammatory response of bone marrow dendritic cells
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Background and aims: Dendritic cells (DC) provide the first line of defence against invading pathogens and play a key role in facilitating cross-talk between the innate and adaptive immune systems. High-fat diet (HFD) induced obesity and insulin resistance (IR) is associated with a heightened inflammatory state and infiltration of macrophages and T-cells into adipose tissue. In this study we hypothesized that a HFD would result in recruitment of DC into adipose tissue, activate the DC immune response, with functional effects on adipocyte insulin sensitivity.
Materials and methods: C57BL/6J mice were fed a HFD (45% palm oil) for 16 weeks, glucose tolerance was monitored pre- and post-HFD. Stromal vascular fraction (SVF) cells were isolated from adipose and analyzed by flow cytometry for $CD11c^+CD11b^+F480^-$ DC. Bone-marrow DC (BMDC) were isolated and stimulated ± LPS (100ng/ml) for 24h and cytokine release measured by ELISA. To characterize the effects of DC on adipocyte biology 3T3-L1 adipocytes were co-cultured with DC (± LPS stimulation), then insulin-stimulated ^{3}H-glucose uptake and insulin signaling was monitored.
Results: Mice developed overt insulin resistance after 16 wks HFD with marked delay in clearance of plasma glucose during GTT compared with chow-fed and week 0 control mice. DC infiltration into adipose tissue was evident after high fat feeding. BMDC derived from HFD-fed mice exhibited a much more pronounced inflammatory response, with greater IL-12p70, IL-10, and IL-1β secretion, in response to LPS compared to age-matched chow-fed control cells. BMDC TLR4 mRNA and protein expression was enhanced in these cells pre-LPS stimulation. Co-culture of BMDC with 3T3L1 adipocytes induced marked insulin resistance in adipocytes with marked reduction in insulin-stimulated ^{3}H-glucose uptake.
Conclusion: HFD induces IR which is associated with infiltration of DC into adipose. BMDC from these HFD fed animals are primed to be more responsive to inflammatory stimuli and can block insulin mediated glucose uptake in adipocytes.
Supported by: SFI (Principal Investigator Programme)

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Inflammatory adipocyte activation by heat shock protein 60 involves MAP-kinase- and NFkB-dependent signalling pathways
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Background and aims: Adipocytes and their mediators have been recognized to play central roles in the development of the metabolic syndrome and in the pathogenesis of diabetes. Recent studies in the New Zealand obese (NZO) mouse, an animal model of the metabolic syndrome, identified heat shock protein 60 (Hsp60) as an endogenous stress signal with pronounced adipocyte stimulating capacity. Hsp60 was found to induce the release of proinflammatory cytokines and chemokines from adipocytes in a receptor-mediated process. With regard to the development of intervention strategies aiming at the modulation of adipocyte-driven proinflammatory processes, our present study was designed to identify key components of signalling pathways involved in the Hsp60-induced release of inflammatory adipocyte mediators.
Materials and methods: Hsp60-mediated activation (phosphorylation) of MAP-kinase family members (p38, ERK1/2, JNK) and of the transcription factor NFκB was analysed by immunoblotting lysates of primary NZO mouse-derived preadipocytes and mature adipocytes. The potential contribution of these signalling molecules to the Hsp60-induced release of the inflammatory mediators IL-6, KC and MCP-1 was assessed by applying specific inhibitors and the use of multiplex beads analyses.
Results: We could demonstrate for the first time that the stress protein Hsp60 activates members of the MAP-kinase family and the transcription factor NFkB in primary adipocytes of the NZO mouse. Hsp60 exposure (10 μg/ml) slightly activated ERK1/2 phosphorylation in mature adipocytes (1.4±0.4-fold), but not in preadipocytes. JNK was weakly activated in mature

adipocytes as well as in preadipocytes up to 2.2±1.2-fold. No Hsp60-induced activation of p38 was observed in the cells. NFkB activation was significantly increased (up to 2.6±0.9-fold) in Hsp60 exposed adipocytes independent of their maturation state ($p<0.05$). The ERK1/2-inhibitor PD98059 suppressed the release of the inflammatory mediator MCP-1 depending on the maturation state of the adipocytes. In preadipocytes the MCP-1 release was slightly decreased in a dose-dependent manner from 5.3±2.0 ng/ml to 3.6±1.4 ng/ml (inhibition by 22.1%), whereas in mature adipocytes the MCP-1 release was strongly reduced from 8.3±5.1 ng/ml to 1.9±2.3 ng/ml (inhibition up to 80.8%, $p<0.001$). The release of IL-6 and KC was not affected by the inhibitory drug. Selective inhibition of JNK by SP600125 caused a weak inhibition of KC-release from mature adipocytes and of MCP-1 release from preadipocytes and mature adipocytes. Inhibition of NFkB activation by SN50 resulted in a strong, dose-dependent reduction of the release of IL-6 (up to 99.9% inhibition, $p<0.001$), KC (up to 91.8% inhibition, $p<0.001$) and MCP-1 (up to 94.1% inhibition, $p<0.01$) from adipocytes independent of their maturation state.

Conclusion: The results of our study point to the involvement of different signalling pathways in the proinflammatory activation of NZO mouse-derived adipocytes by Hsp60. Whereas ERK1/2 and JNK are differentially activated depending on the adipocyte maturation state, NFκB activation is largely independent of the maturation state of the cells. Our findings will contribute to identify components of the intracellular signalling cascades as potential targets for the modulation of diabetes-associated inflammatory adipocyte activities induced by endogenous stress signals.

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Short chain fatty acid regulation of inflammatory response in human primary macrophages

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Background and aims: Insulin resistance in adiposity and type 2 diabetes is associated with low grade system inflammation characterized by macrophage infiltration of adipose tissue. Short chain fatty acids (SCFA) produced by intestinal microbiota modulate inflammatory responses. This effect have been demonstrated in various immune cells (neutrophiles, monocytes, PBMC fraction), tumor cell lines, tissue culture and animal studies, but not in mature human macrophages. The aim of this study was to investigate SCFA effects on the cytokine expression in human primary macrophages of proinflammatory M1- and antiinflammatory M2-subtypes.

Materials and methods: For the culture of human primary macrophages, monocytes were isolated from the whole blood and differentiated in RPMI medium supplemented with 50 ng/ml GM-CSF (M1-macrophages) or M-CSF (M2-macrophages) and 10% foetal bovine serum for 7 days. For the experiment, mature cells were incubated in the medium with acetate or propionate (2, 20 mM) for two hours with following LPS treatment (100 ng/ml) for 22 hours. The expression of cytokines (MCP1, IL-1β, IL-6, IL-8, IL-10, TNF-α) and SCFA receptors was assessed by quantitative real-time PCR.

Results: The low level of mRNA expression of SCFA receptors GPR41 and GPR43 activated by acetate and propionate was detected in both studied subtypes of human primary macrophages. Interestingly, LPS treatment significantly increased the expression of both receptors with this effect being more apparent in M2-macrophages (219-fold for GPR41 and 46-fold for GPR43). Acetate (20 mM) and propionate (2, 20 mM) treatment considerably attenuated LPS-induced receptor expression. SCFA effects on the basal and LPS-induced cytokine expression were studied. Propionate at high concentration (20 mM) corresponding to the concentration in human colon demonstrates antiinflammatory effect due to significantly decrease of the LPS-induced cytokine expression. Interestingly, the same propionate treatment increased cytokine expression in macrophages incubated without LPS stumulation. These effects were detected both in M1- and in M2-cell subtypes. For acetate, no influence on the cytokine expression was found.

Conclusion: Propionate demonstrates modulatory effects on the inflammatory response in the culture of human primary macrophages which are possibly mediated by metabolic, rather than receptor-coupled mechanism. Thus, propionate produced by intestinal microbiota may contribute to the regulation of inflammatory responses of tissue macrophages in adiposity and type 2 diabetes.

Supported by: DFG

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Acute and chronic saturated fatty acid treatment as a key instigator of the TLR mediated inflammatory response in human adipose tissue *in vitro*

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Background and aims: Chronic elevation of saturated fatty acids (SFAs) and glucose (Glc) appears to activate an inflammatory response; compounded by habitual feeding. Restoration of physiological SFAs and Glc levels postprandially may not attenuate the original insult; a concept termed 'metabolic memory'. Therefore we investigated (1) the effect of chronic and oscillating SFAs and Glc on the inflammatory pathway in human abdominal subcutaneous (AbdSc) adipose tissue (AT) and adipocytes (Ads) (2) whether there is a sustained inflammatory response in absence of treatment.

Materials and methods: AbdSc AT (age 45±3.3 yrs; BMI: 21.9±2.4 kg/m^2; n=6) was obtained with ethics approval. Explants and Ads were treated with chronic low glucose (L-Glc): 5.6mM and high glucose (H-Glc): 7.5mM, with low (0.2 mM) and high (2 mM) doses of a palmitate:stearic tri-mix (SFA) for 48 hrs. Further, AbdSc AT explants and Ads were also exposed to the aforementioned treatment regimen for 12hr periods, with alternating rest periods of 12hr in L-Glc. Western blot and ELISAs were utilised to examine components of the NFκB pathway.

Results: Chronic treatment with H-Glc and high SFAs up-regulated key proteins of the NFκB pathway in AbdSc AT explants and Ads (TLR4, NFκB, $p<0.05$) whilst down-regulating MyD88 protein levels. Oscillating treatment with Glc and SFAs increased TLR4, NFκB, IKKβ and MYD88 in explants and Ads ($p<0.05$). Downstream TNFα and IL-6 ($p<0.05$) secretion were markedly increased in chronically treated explants and Ads whilst, with oscillating treatments, a sustained inflammatory effect was noted in absence of treatment.

Conclusion: In summary, this study implicates elevated SFAs as a key instigator of the inflammatory response in both AT and Ads, via NFκB, and suggests that short-term exposure of cells to uncontrolled levels of SFAs and Glc leads to a longer-term inflammatory insult within the Ads. As such, these findings highlight a potential molecular mechanism linking high SFA dietary intake and an adverse inflammatory response in patients with obesity and T2DM.

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Distinct gene expression and cytokine release profiles identify three different adipose tissue progenitor cells in human abdominal subcutaneous and visceral adipose tissue

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Background and aims: Adipose-derived stem cells (ASCs) can be isolated from the stromal-vascular fraction (SVF) of adipose tissue (AT) (ASC-SVF). ASCs can be also isolated both from a pre-adipocyte fraction present in the fat cake at the top of the supernatant not previously collected together with the ASC-SVF (ASC-B), and from mature adipocytes through in vitro de-differentiation (ASC-C). The aim of this study was to investigate the biological features of these three different ASCs.

Materials and methods: All ASCs were isolated from paired abdominal subcutaneous (SC) and visceral (V) AT biopsies obtained from 16 subjects (8F/8M, age 65±1 yrs, BMI 24.9±1.4). A genome-wide differential gene expression analysis followed by quantitative RT-PCR was performed on each SC and V ASC population. Cytokine secretion was analyzed by Bioplex protein array of the ASC conditioned medium.

Results: The six distinct ASCs were positive for the adipogenic lineage markers CD105, CD44, and CD49d, and were able to differentiate into adipocytes, chondrocytes, and osteocytes, respectively. To investigate whether ASC-SVF, ASC-B and ASC-C from SC and V fat depots were homogenous or genetically heterogeneous, a genome-wide analysis was performed using a GeneChip Gene 1.0 ST array with 764,885 probe sets, representing 28,869 annotated genes. Of these, we identified 367 and 984 genes that differed among ASC-SVF, ASC-B and ASC-C in SC and V, respectively. These genes were identi-

fied by first testing for significant differences among all ASCs in the 764,885 probe sets with sequence-specific hybridization in at least one sample (Bayesian ANOVA with a P value <0.05). A secondary filter was applied to those genes that resulted significantly different, to identify the genes that varied by at least 1.4-fold among V and SC ASCs. In SC ASCs, of the 367 genes, 169 were found to have a conjoint differential expression in ASC-SVF, ASC-B and ASC-C, while 16, 76 and 106 were the genes that resulted to be differentially expressed in each subset of ASC, respectively. In addition, no gene was found to have a conjoint differential expression in SC ASC-SVF, ASC-B and ASC-C. In V ASCs, 984 genes were found to be differently expressed among ASC-SVF, ASC-B and ASC-C. Of these, 230 genes were found to have a conjoint differential expression in each V ASC population, while 235, 426 and 89 were the genes that resulted to be differentially expressed in each subset of ASC, respectively. In addition, 4 genes resulted to have a conjoint differential expression in all V ASCs. Both in SC and V ASCs, hierarchical clusters identified the presence of large contiguous color patches representing groups of genes that shared a similar expression pattern over multiple conditions both in ASC-SVF and ASC-C, while highly dissimilar was the gene expression pattern in ASC-B compared to ASC-SVF and ASC-C. Of the 26 cytokines measured, IL-6 was secreted at higher levels in SC ASC-C compared to SC ASC-SVF and ASC-B ($p<0.01$), while it was significantly lower in SC ASC-SVF and ASC-B than in V ASCs ($p<0.01$). Secretion of IL-8, VEGF and MCP-1 was also significantly different among the 6 SC and V ASCs.
Conclusion: These results demonstrate the existence of genetically heterogeneous fat-derived populations of progenitor cells, which may add to the complexity and specificity of SC and V AT in humans.
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Plasma glucose levels are associated with gene expression levels in subcutaneous and visceral adipose tissue of morbidly obese individuals
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Background and aims: Morbid obesity (BMI>35) dramatically increases the risk to develop type 2 diabetes. The biological mechanisms that drive this increased risk are largely unclear. In this study we aimed to identify genes that are differentially expressed in subcutaneous and visceral adipose tissue of diabetic and non-diabetic obese individuals in order to get insight in the pathologic changes related to diabetes.
Materials and methods: We determined whole-transcriptome gene expression levels in subcutaneous adipose tissue and visceral adipose tissue of 70 morbidly obese individuals by using microarrays (Illumina HumanHT12 BeadChips). From these data we extracted modules of highly coexpressed genes and we tested for each tissue whether these modules correlate with traits that are relevant to diabetes (plasma levels of glucose, insulin, and HbA1c). These modules are driven by the phenotypic differences in our study group, which range from metabolically normal to diabetic.
Results: In both tissues we detected several modules of highly coexpressed genes. Gene Set Enrichment Analysis of the genes within all separate modules showed that the genes within most modules are functionally related. This indicates that our approach yields a biologically meaningful gene classification. Assessment of the correlation between each module and metabolic traits related to diabetes, revealed that in subcutaneous adipose tissue one module is significantly negatively correlated to plasma glucose levels ($p = 7.0 \times 10^{-6}$). This module consists of 28 genes of which most are involved in metabolism. In visceral adipose tissue one module - consisting of 103 genes - is significantly correlated to plasma glucose levels ($p = 2.1 \times 10^{-5}$). Some genes that constitute this module were recently reported to be specifically expressed in macrophages, and are highly enriched in genes related to innate immunity (GO-terms).
Conclusion: In both subcutaneous and visceral adipose tissue we identified several genes that have expression levels which are correlated with plasma glucose levels. In subcutaneous adipose tissue expression levels of a set of metabolic genes were inversely correlated with plasma glucose levels. In visceral adipose tissue a set of genes related to innate immunity were positively correlated to plasma glucose levels. In particular, these genes play a role in the complement system (C1QA, C1QB, C1QC, C3AR1), Toll-like receptor signaling (TLR7, CD14), inflammasome (PYCARD), autophagy (ATG7), and lysosomal function (CTSB, CTSC, CTSZ, FUCA1, GUSB, LGMN). In conclusion, our data indicate that both metabolic and innate immunity related processes in subcutaneous and visceral adipose tissue, bridge the gap between diabetes and obesity.
Supported by: DFN

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Effect of hyperinsulinaemia on selected plasma and subcutaneous adipokines during angiotensin II type 1 receptor inhibition in patients with impaired fasting glucose
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Background and aims: Only a few studies have investigated the in vivo effect of insulin on adipokines during angiotensin II type 1 receptor inhibition. The aim of our study was to test the effect of insulin on selected plasma and subcutaneous adipokines and their insulin-stimulated changes during telmisartan administration.
Materials and methods: 12 patients with impaired fasting glucose were enrolled in randomized, placebo-controlled, cross-over study of 3 weeks treatment with telmisartan (T) (160 mg/d) or placebo (P). Acute hyperinsulinemia has been induced by one-step hyperinsulinemic euglycemic clamp (120 minutes; 1 $mU.kg^{-1}.min^{-1}$, 5 mmol/l) conducted at the end of each treatment period. Before and during the clamp (0, 30, 120 minutes) plasma levels of adiponectin, resistin, tumor necrosis factor-alfa (TNF) and leptin were measured and needle biopsy of abdominal subcutaneous adipose tissue (SAT; 0, 30 minutes) was performed to evaluate their expressions by the real-time PCR.
Results: Hyperinsulinemia did not affect plasma TNF, but decreased TNF expression ($p<0.001$). Plasma adiponectin concentrations and SAT expression of adiponectin did not changed during insulin-stimulated conditions. Hyperinsulinemia did not change plasma leptin concentrations, but the decrease in leptin expression in SAT was found ($p<0.001$). Plasma resistin rise during hyperinsulinemia ($p<0.001$), while the drop in resistin expression was observed ($p<0.01$). Telmisartan decreased plasma TNF ($p<0.05$) and increased plasma leptin ($p<0.01$) and resistin ($p<0.001$) as compared to P. Moreover, telmisartan increased the plasma adiponectin ($p<0.05$), leptin ($p<0.05$) and resistin ($p<0.01$) concentrations during hyperinsulinemia.
Conclusion: Acute hyperinsulinemia increases plasma resistin concentrations and decreases resistin, leptin and TNF expressions in SAT. Telmisartan decreases plasma TNF and increases plasma leptin and resistin concentrations and changes the effects of insulin on plasma adiponectin, leptin and resistin concentrations. Our results support the hypothesis that telmisartan might play the beneficial metabolic role in patients with impaired fasting glucose.
Supported by: MZO

PS 72 Animal models of obesity and/or insulin resistance

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Absence of the NLRP-3 inflammasome protects against the development of obesity and insulin resistance

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Background and aims: The innate immune system is part of the host defence and responds to invading pathogens by inducing an inflammatory reaction. Recognition of pathogens depends on PRRs that are present both on the outer membrane and intracellular components. The nucleotide-binding and oligomerization domain (NOD)-like receptor (NLR) protein NLRP3 responds to microbial ligands and endogenous danger signals present in the cytosol and triggers the formation of a protein complex including the eminent adaptor molecule ASC. The protein complex is named the NLRP-3 inflammasome and, upon formation, it enables the activation of Caspase-1, a cysteine protease that controls release of the pro-inflammatory cytokines IL-1β and IL-18. Obesity is characterized by elevated secretion of numerous cytokines including IL-1β and IL-18 that contribute to the development of insulin resistance and type 2 diabetes. Previously we have shown that Caspase-1 is activated in adipose tissue of obese and insulin resistant animals, which suggests that the innate immune system is involved in the development of metabolic abnormalities associated with obesity. In the present study we tested whether (NLRP-3) inflammasome-dependent Caspase-1 activation mediates high fat diet-induced obesity and insulin resistance.
Materials and methods: To induce obesity and insulin resistance, Wildtype (Wt), NLRP3-/-, ASC-/- and Caspase-1-/- animals were fed a high fat diet (HFD) or low fat diet (LFD) for 16 weeks.
Results: Despite a similar daily food intake, HFD-fed NLRP3-/-, ASC-/- and Caspase-1-/- animals were protected against the development of high fat diet induced obesity**.** Whereas HFD-feeding of wildtype mice led to significantly elevated plasma insulin, leptin and resistin levels, animals lacking the NLRP3-inflammasome were protected against the harmful effects of chronic overfeeding. Importantly, ASC-/- and Caspase-1-/- animals were resistant to the development of high-fat diet induced insulin resistance. In addition to a decrease in total adipose tissue mass, adipocyte cell size was reduced in HFD-fed ASC-/- and Caspase-1-/- animals compared to Wt mice. Detailed analysis of HFD-fed Caspase-1-/- mice using immunohistochemical localization of adipose tissue-resident macrophages revealed a robust reduction of macrophage influx into the adipose tissue. Using microarray analyses of white adipose tissue from HFD-fed Wt vs. Caspase-1-/- animals, we established that over 500 genes involved in immune response, signal transduction and chemotaxis were differentially expressed. Finally, metabolic cage studies of HFD-fed Wt and Caspase-1-/- animals revealed an enhancement in total energy expenditure in the absence of Caspase-1.
Conclusion: Our results show that NLRP-3 inflammasome-mediated caspase-1 activity is involved in the development of obesity, insulin sensitivity, adipogenic gene expression and energy expenditure during chronic overfeeding and suggest that inhibition of inflammasome-dependent caspase-1 activation may be a useful therapeutic strategy for treatment of obesity-induced insulin resistance.
Supported by: The Dutch Diabetes Research Foundation

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Deletion of macrophage migration inhibitory factor promotes obesity-associated insulin resistance while attenuating inflammation in mice fed a high-fat diet

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Background and aims: Macrophage migration inhibitory factor (MIF) is a multifunctional molecule generally described as pro-inflammatory and glucocorticoid-induced regulator involved in both innate and adaptive immunity. MIF is produced by various immune and non-immune cells including pancreatic β cells. Elevated MIF concentration and MIF mRNA expression was found in the mononuclear cells of obese individuals and serum MIF concentrations are highly increased in individuals with impaired glucose tolerance and type 2 diabetes. Furthermore, an increased expression of MIF mRNA and its protein in pancreatic islets isolated from high-fat diet (HFD)-fed C57BL/6 mice (B6) compared to control diet (CD)-fed mice has been seen (unpublished). Also, increased MIF levels were found in serum of starved animals on HFD. Since obesity is characterized as a state of low grade inflammation, the aim of this study was to investigate the effect of *mif* gene deletion on development of obesity, insulin resistance and inflammatory state in HFD-fed mice.
Materials and methods: Knock-out mice for *mif* gene (MIF-KO) and their wild type counterparts B6 mice were fed a HFD containing 60% fat and control groups were fed a control diet (CD - 10% fat). The weight of mice was measured weekly, triglyceride levels in non-fasting mice were determined spectrophotometrically, while serum markers of inflammation and insulin were measured by ELISA. Glucose and insulin tolerance tests were performed on fasting animals by intraperitoneal injection of D-glucose (2 mg/g b.w) or insulin (0.75 mU/g), respectively. Glucose concentration was determined from blood drawn from tail vein by Glucometer.
Results: Even on standard chow MIF-KO mice gained more weight than B6 mice on the same diet. Furthermore, body mass increment of MIF-KO on HFD was higher compared to HFD-fed B6 mice. As for triglyceride levels, they were similar between MIF-KO and B6 when mice were on CD. Interestingly, although MIF-KO on HFD diet weighed more then B6 on the same diet, their triglycerides were lower, at least at some time points tested. On the other hand, leptin levels corresponded to the weight increase of HFD-fed MIF-KO. Similar values of hyperglycemia were found in both HFD-fed B6 and MIF-KO mice. Although MIF-KO mice on CD were euglycemic, they showed slightly impaired glucose and insulin tolerance. Moreover, marked insulin resistance (judged by both glucose and insulin tolerance test) was evident in HFD-fed MIF-KO mice and was significantly altered compared to B6 mice on HFD. Serum insulin in MIF-KO on HFD was higher compared to CD-fed mice and similar to B6 mice on HFD. Finally, at the end of observation period (11 weeks), inflammatory markers such as CRP and IL-6 were down-regulated in HFD-fed MIF-KO compared to B6, while anti-inflammatory cytokine TGF-β was moderately increased.
Conclusion: These results implicate that *in vivo* MIF deletion exacerbates obesity-induced insulin resistance, but reduces an underlying inflammation during high nutrient intake.
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Expression of human chemerin induces insulin resistance in the skeletal muscle but does not affect weight, lipid levels and atherosclerosis in LDL receptor knockout mice on high fat diet

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Background and aims: Chemerin is a recently discovered hepatoadipokine that regulates adipocyte differentiation as well as chemotaxis and activation of dendritic cells and macrophages. Chemerin was reported to modulate insulin sensitivity in adipocytes and skeletal muscle cells *in vitro*. In humans, chemerin was shown to be associated with multiple components of the metabolic syndrome including body mass index, triglycerides, HDL-cholesterol and hypertension. So far, however, the effect of chemerin on these various metabolic parameters has not been studied *in vivo*.
Materials and methods: To investigate the effect of chemerin on weight, glucose and lipid metabolism as well as atherosclerosis *in vivo*, we used recombinant adeno-associated virus to express human chemerin in LDL receptor knockout mice on high fat diet.
Results: Expression of chemerin did not significantly alter weight, lipid levels, and extent of atherosclerosis. Chemerin, however, significantly increased glucose levels during both intraperitoneal glucose tolerance test and insulin tolerance test. Chemerin reduced insulinstimulated Akt1 phosphorylation and activation of 5´AMP activated protein kinase (AMPK) in the skeletal muscle, but had no effect on insulin signaling and AMPK activation in the liver and gonadal adipose tissue.
Conclusion: Chemerin induces insulin resistance in the skeletal muscle *in vivo*. Chemerin is involved in the cross talk between liver, adipose tissue and skeletal muscle.

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Adipose tissue-specific activation of polyamine catabolism improves energy homeostasis in mice

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Background and aims: Polyamines are cationic and water-soluble compounds found in all eukaryotic cells. A well-known function of polyamines is their ability to promote cell growth but all their functions are not known. Our previous study in transgenic mice having whole-body overexpression of the key enzyme in polyamine catabolism, spermidine/spermine-N^1-acetyltransferase (SSAT), showed that enhanced polyamine catabolism regulates glucose and energy metabolism. The aim of this study was to investigate the effect of adipose tissue-specific activation of polyamine catabolism on glucose and energy metabolism in mice.
Materials and methods: We generated a transgenic mouse line (aP2-SSAT mice) overexpressing SSAT under an adipose tissue specific aP2 promoter. The SSAT activity was analyzed in a reaction where [14C]-acetyl-CoA was incorporated into polyamines. Basal metabolic rate was determined by indirect calorimetry. Both aP2-SSAT and wild type mice were challenged with high-fat diet providing 42 % calories from fat for three months. AMPK (AMP kinase) and PGC-1 α (peroxisome proliferator-activated receptor γ co-activator 1 α) levels in WAT were determined by western blotting. Glucose metabolism in wild type (wt) and transgenic (tg) mice was studied using intraperitoneal glucose and insulin tolerance tests (GTT and ITT). Plasma glucose levels were determined microfluorometrically and plasma insulin levels with a commercial insulin kit.
Results: SSAT enzyme activity was significantly higher in white (wt: 0.19 ± 0.03 vs. tg: 17.69 ± 2.42 pmol/μg DNA/10 min, p<0.0001) and brown (wt: 0.08 ± 0.05 vs. tg: 1.84 ± 0.46 pmol/μg DNA/10 min, p<0.005) adipose tissues in aP2-SSAT mice than in wt mice. aP2-SSAT mice had significantly reduced perigonadal WAT mass (wt: 2.28 ± 0.16 vs. tg: 1.65 ± 0.15 % of body weight, p<0.005), and white adipocytes had an increased amount of mitochondria. In addition, aP2-SSAT mice had significantly greater oxygen consumption in comparison with wt mice (wt: 1.81 ± 0.05 vs. tg: 2.02 ± 0.06 VO_2 ml/min/100g, p<0.05) implicating that energy expenditure is enhanced in aP2-SSAT mice. When challenged with high-fat diet (HFD), aP2-SSAT mice were resistant to HFD-induced body weight gain (wt control vs. wt HFD body weight p<0.005; tg control vs. tg HFD body weight p>0.05). These findings were explained by increased protein levels of the key regulators of energy metabolism, PGC-1α (wt: 0.68 ± 0.27 vs. tg: 1.86 ± 0.22, p<0.05) and AMPK (wt: 0.94 ± 0.17 vs. tg 1.94 ± 0.25) in WAT of aP2-SSAT mice. Based on glucose and insulin tolerance tests, no changes in glucose metabolism were observed (GTT; wt: 1123.00 ± 114.00 vs. tg: 1237.00 ± 69.48 area under the curve for plasma glucose, p>0.05; GTT; wt vs. tg plasma insulin levels p>0.05 and ITT; wt vs. tg relative glucose levels p>0.05).
Conclusion: Our results suggest that adipose tissue-specific activation of polyamine catabolism does not improve glucose homeostasis but causes beneficial changes in energy metabolism.
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Anti-diabetic effect of sodium tungstate in IRS2-deficient mice model

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Background and aims: The insulin receptor substrate-2 (IRS2) branch of the insulin/IGF signalling mediates peripheral insulin action and plays an essential role in pancreatic β-cell function and survival. In fact, IRS-2 is a key molecule in the control of beta cell mass and is directly phosphorylated by the insulin receptor after insulin binds the latter, leading to the recruitment and activation of additional signalling proteins. Ablation of the IRS-2 gene in mice results in a phenotype with characteristics of human type-2 diabetes - they progress toward diabetes as beta cell mass decreases and insulin secretion fails. Sodium Tungstate could be involved in the reversion of the diabetic phenotype observed by acting independently of the insulin signalling pathway. In the present work, we used IRS2 knockout mice and examined the effects of tunsgtate administration in order to check for its potential targets as well as its therapeutic potential.
Materials and methods: 10 weeks wild type and knockout IRS2 C57Bl/6 mice were divided into two groups, untreated and treated with a solution of 2mg/ml of sodium tungstate in distilled water. After 21 days metabolic studies such as glucose tolerance tests and morphometric analysis of pancreatic sections by immunofluorescence techniques were performed in order to identify the effects of a tungstate treatment.
Results: Our results illustrated that tungstate administered orally normalised blood glucose concentration in IRS2 knockout, despite the initial high glycaemic values (fasting initial values: 221±26 mg/dl compared to 139±23 mg/dl at day 21). This was accompanied by an improvement of glucose tolerance of IRS-2 $^{-/-}$ treated versus untreated group, assessed by an intraperitoneal glucose tolerance test performed both before and after treatment (figure). Morphometric analysis of pancreatic sections of these animals revealed as expected a reduced total islet and beta cell area in the untreated IRS-2 $^{-/-}$ mice (45295um^2±29239 and 38402um^2±25014 respectively) when compared to wild-type animals (145722um^2±78299 and 137357um^2±77630); however, treated knockout mice showed comparable values (140211um^2± 84245 and 121277um^2± 74254).
Conclusion: Our results indicate that sodium tungstate may have an anti-diabetic effect in IRS-2 knockout mice and could help us understand the molecular mechanisms underlying the regulation of glucose homeostasis and endocrine plasticity.

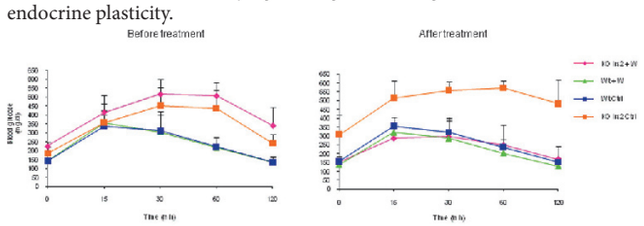

Figure. Glucose-tolerance tests after intraperitoneal loading with 2 g D-glucose per Kg were performed on 10-week-old animals of the indicated genotype

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The effects of mildronate and metformin on energy metabolism pathways in experimental model of obesity

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Background and aims: Mildronate is a cardioprotective drug, which mechanism of action is based on the regulation of carnitine concentration. In addition to its cardioprotective effects mildronate lowers blood glucose concentration and protects against diabetes complications in Goto-Kakizaki rats. The present study was carried out to investigate the metabolic effects of mildronate, metformin and a combination of the two in the experimental Zucker rat model of obesity and impaired glucose tolerance.
Materials and methods: Zucker rats were perorally (p.o.) treated daily with mildronate (200 mg/kg), metformin (300 mg/kg), and a combination of both drugs for 4 weeks. Weight gain and plasma metabolites reflecting glucose and lipid metabolism were measured by commercially available kits. The amounts of PPAR-alpha and PPAR-gamma as well as their target gene expression in heart and liver tissues were detected by Western blot and qRT-PCR analysis, respectively.
Results: Both tested drugs and the combination similarly decreased fed and fasted state blood glucose by 1-2 mmol/l. In addition, mildronate, metformin and the combination significantly decreased fed state plasma insulin concentration for 31%, 29% and 47%, respectively. Mildronate significantly stimulated the expression of PPAR-alpha in the heart and PPAR-gamma in the heart and liver. Also the increased expression of PPAR-alpha target genes in the heart, but not in liver tissues was observed. In contrast to monotherapy, treatment with the combination of mildronate and metformin significantly decreased the weight gain for 19%, while it did not affect food intake.
Conclusion: In conclusion, our results demonstrate that mildronate, an inhibitor of L-carnitine biosynthesis, enhances the anti-diabetic activity of metformin, mediates PPAR-alpha and PPAR-gamma activation and improves the adaptive responses against hyperglycemia- and hyperlipidemia-induced metabolic disturbances.
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Analysis of spontaneous obese and diabetic mice induced by selective breeding with high fat diet

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Background and aims: Diabetes and obesity become more common due to modern lifestyle as environmental factor, and familial clustering as genetic factor is indicated in such a dysmetabolism. To elucidate the influence of present lifestyle to the next generation, we performed selective breeding of mice that had glucose dysmetabolism induced by high fat diet (HFD).
Materials and methods: Hybrid mice with male C57BL/6J, female C3H/HeJ and female AKR were used in this study. At first, male C57BL/6J mice were crossed with female C3H/HeJ mice, and mice were fed with 25% HFD for 10 weeks (5-15 weeks of age). Then casual blood glucose was checked and mice with higher blood glucose level were selected for breeding. At the 3rd generation, birth rate was reduced, so we crossed these male mice with female AKR mice, and fed these mice with 13.5% HFD for 10 weeks. From the 4th generation to date, oral glucose tolerance test (OGTT) was performed around 11 weeks old, and mice with higher blood glucose levels at 2 hours (2hr BG) were selected and bred as high glucose colony, H-strain. In the present protocol, mice were fed with 13.5% HFD from 5 to 10 weeks old to keep the normal birth rate. Despite HFD feeding, some of mice showed normal GTT pattern. These mice were also selected and bred as control (normal glucose colony; C-strain). Messenger RNA expression of several genes in liver, skeletal muscle, and fat tissues, which influence glucose metabolism and body weight, was evaluated by real time quantitative PCR at 5 weeks old before HFD feeding.
Results: Now we have maintained the 12th generation of H-strain and the11th of C-strain. At 10 weeks old (after HFD feeding), the frequency of IGT and DM (2hr BG level is 140-199 mg/dl and over 200 mg/dl, respectively) was significantly higher in H10 (96.4% in male and 45.0% in female) than C9 (9.1% and 0%, respectively) by chi-square test. Body weight gain after HFD feeding was accelerated in H-strain compared to C-strain, and increased to 32.3±3.1 vs 29.2±2.4 g (male, P=0.0003) and 24.0±1.9 vs 20.7±1.4 g (female, P<0.0001), respectively. There was no difference in food intake between the two groups. At 5 weeks old (before HFD feeding), 2hr BG level of OGTT was 161±42.6 vs 105±25.3 mg/dl (H vs C-strain of male, P=0.042) and 112±34.1 vs 67±12.2 mg/dl(H vs C strain of female, P=0.046), therefore H-strain already had abnormal glucose metabolism before fat load. Real time PCR analysis of 5 weeks old male mice showed that UCP-2 expression of liver and muscle was significantly elevated, and UCP-1 / -3 expressions of BAT were significantly decreased in H-strain compared to C-strain.
Conclusion: Abnormal glucose metabolism and obesity induced by HFD were accelerated by selective breeding, thus the susceptibility to HFD was transmitted to next generation. It is concluded that the new strain of obese and diabetic mice was established. Additionally, further analysis of these mice could reveal the genetic factors that regulate sensitivity to HFD.

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***In vivo* efficient gene transfer to murine white adipose tissue using adeno-associated viral vectors**

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Background and aims: Obesity is a worldwide growing health problem and this alteration of the metabolic and endocrine functions of adipose tissue is frequently associated with insulin resistance and type 2 diabetes. In order to increase our knowledge about the molecular mechanisms that underlie obesity, the overexpression or knockdown/silencing of specific genes in adipose tissue may offer great potential. However efficient gene transfer postnatally into adipocytes in vivo has not been achieved. Therefore, this study was designed to test the ability of adeno-associated viral vectors (AAV) serotypes 1, 2, 4, 5, 6, 7, 8 and 9 to achieve broad, efficient and persistent gene transfer to murine white adipose tissue in vivo.
Materials and methods: AAV vectors coding GFP as a marker gene or the enzyme Hexokinase II (HKII) were generated by triple transfection in 293 cells and were purified by double cesium chloride gradient. AAV vectors were injected into the epididymal white adipose tissue of mice.
Results: To asses the in vivo transduction efficiency of the adipose tissue with adeno-associated viral vectors, AAV serotypes 1, 2, 4, 5, 6, 7, 8 and 9 encoding the marker protein GFP were injected into the epididymal white adipose tissue (eWAT) of mice. Two weeks after the injection, fat pads treated with AAV8 and AAV9 presented the highest GFP content that paralleled the highest numbers of transduced adipocytes indicating that these two serotypes were the most efficient transducing adipocytes in vivo. In addition, to examine whether in vivo AAV-transduced adipocytes may be a viable model to study adipose function, differentiation and metabolism, gene transfer of key metabolic genes such as HKII into eWAT was also evaluated. Two weeks after injection, isolated adipocytes from mice injected with AAV9 vectors coding HKII presented a 3-fold overexpression of HKII mRNA compared with adipocytes from AAV9 null-injected mice. In AAV9-HkII-transduced adipocytes, insulin produced a marked increased in the 2-[1-3H]deoxy-D-glucose uptake compared to AAV9 null-transduced adipocytes at low and maximal insulin concentrations in a dose dependent manner.
Conclusion: All together, these results show that AAV vectors may be very useful for genetic modification of adipose cells in vivo to analyze adipocyte function or to assay new gene therapy approaches targeting the adipose tissue.
Supported by: SAF2008-00962 and the EC

PS 73 DPP IV inhibitors

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Sitagliptin and metformin increase active GLP-1 by complementary mechanisms in treatment-naïve patients with type 2 diabetes

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Background and aims: Sitagliptin (SITA) and metformin (MET) are oral antihyperglycemic agents with different, complimentary mechanisms-of-action. Because treatment of type 2 diabetes mellitus (T2DM) usually requires combination therapy we assessed the potential combination effects of SITA and MET on GLP-1 in patients with T2DM.
Materials and methods: This was a randomized, placebo (PBO)-controlled, double-blind, 4-period crossover study in 18 treatment-naïve patients with T2DM. In each 2-day period, subjects received either SITA 100 mg in AM on Days 1 and 2, MET 500 mg in AM and PM on Day 1 and 1000 mg in AM on Day 2, co-administration of SITA+MET on Days 1 and 2 or PBO on Days 1 and 2. On Day 2, at 2 hrs post-AM dose, patients ate a standard meal. Blood samples were collected pre- and at specified times post-meal.
Results: Compared with PBO, SITA, MET, and SITA+MET reduced 2-hr post-meal glucose by 31, 40, and 74 mg/dL, respectively. Compared with PBO, MET alone increased cumulative 4-hr post-meal weighted mean (WM) total GLP-1 levels by 1.5-times while SITA slightly decreased levels by ~10%, consistent with feedback inhibition of GLP-1 release by increased active GLP-1. SITA or MET alone each increased cumulative 4-hr post-meal active GLP-1 levels by 2.2- and 1.7-times, respectively, and in combination by 3.4-times. MET increased active GLP-1 in proportion to the increase in total GLP-1, suggesting that the increase in active was primarily due to the increase in total GLP-1. In contrast, SITA increased active, but not total GLP-1, consistent with stabilization of the active peptide. These data are similar to previously reported results in healthy non-diabetic subjects.
Conclusion: Co-administration of SITA and MET enhances reductions in glucose and increases in active GLP-1, and may provide a unique benefit to patients with T2DM as a result of these complementary mechanisms-of-action.

Treatment	Geometric LS Mean (95% CI)[†]	Ratio of Geometric LS Means (Active/Placebo)(95% CI)
4-hr WM Total GLP-1 (pM)		
SITA	4.98 (4.24, 5.84)	0.87 (0.78, 0.97)
MET	8.73 (7.44, 10.2)	1.52 (1.37, 1.70)
SITA+MET	6.95 (5.93, 8.15)	1.21 (1.09, 1.35)
PBO	5.74 (4.89, 6.73)	---
4-hr WM Active GLP-1 (pM)		
SITA	9.06 (7.82, 10.5)	2.16 (1.87, 2.49)
MET	6.98 (6.02, 8.08)	1.66 (1.44, 1.92)
SITA+MET	14.3 (12.3, 16.6)	3.41 (2.95, 3.93)
PBO	4.20 (3.62, 4.86)	---

[†]Back-transformed from log scale

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Sitagliptin compared with glimepiride provides similar efficacy with weight loss and less hypoglycaemia when added to metformin therapy in patients with type 2 diabetes mellitus

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Background and aims: Current guidelines recommend the addition of a second antihyperglycemic agent when glycemic control is not achieved with metformin monotherapy. Sulfonylureas are the most common antihyperglycemic agents used in combination with metformin among patients who do not achieve or maintain glycemic control on metformin alone. In previous studies, the DPP-4 inhibitor sitagliptin (SITA) significantly improved glycemic control and was well tolerated when added to ongoing metformin monotherapy. The current study compared the efficacy and safety of SITA with the commonly-used sulfonylurea glimepiride (GLIM).
Materials and methods: A randomized, double-blind study was conducted in patients with T2DM who had inadequate glycemic control (A1C of 6.5%-9.0%) while on a stable dose of metformin (≥1500 mg/day for ≥12 weeks). After a 2-week placebo run-in period, 1035 patients were randomized to the addition of SITA 100 mg/day (N=516) or GLIM 1 mg/day (uptitrated to a potential maximum 6 mg/day; N=519) to ongoing metformin monotherapy for 30 weeks. The primary analysis evaluated whether SITA was non-inferior to GLIM in reducing A1C from baseline at Week 30 using a predefined non-inferiority margin of 0.4%.
Results: From a mean baseline A1C of 7.49% across treatment groups, LS mean changes at Week 30 were -0.47% for the SITA group and -0.54% for the GLIM group (between-group difference = 0.07% [95% CI: -0.03, 0.16]). The upper limit of the 95% CI for the between-group difference in change from baseline in A1C (0.16%) was less than the non-inferiority margin of 0.4%, supporting a conclusion of non-inferiority for SITA versus GLIM. The percentages of patients with A1C <7.0% at Week 30 were 52.4% (SITA) and 59.6% (GLIM). LS mean changes from baseline in fasting plasma glucose were -14.6 mg/dL and -17.5 mg/dL for SITA and GLIM, respectively. The percentage of patients for whom at least one adverse event of hypoglycemia was reported was significantly ($p<0.001$) lower with SITA (7%) than with GLIM (22%), with 73 and 460 events, respectively, reported. Treatment with SITA led to mean weight loss (-0.8 kg; 95% CI: -1.1, -0.5) while treatment with GLIM led to mean weight gain (1.2 kg; 95% CI: 0.9, 1.5), with a significant ($p<0.001$) between-group difference of -2.0 kg (95% CI: -2.3, -1.6).
Conclusion: In this study, the addition of SITA compared with the addition of GLIM to ongoing metformin monotherapy in patients with T2DM provided similar A1C-lowering efficacy after 30 weeks. Sitagliptin was generally well tolerated, with a lower risk of hypoglycemia and with weight loss compared with weight gain with glimepiride.
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Efficacy and safety of sitagliptin and the fixed-dose combination of sitagliptin and metformin versus pioglitazone in drug-naïve patients with type 2 diabetes

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Background and aims: Combination therapy has been recommended for patients with type 2 diabetes mellitus (T2DM) who have greater degrees of hyperglycemia. The DPP-4 inhibitor sitagliptin (SITA) and the fixed-dose combination of sitagliptin + metformin (SITA/MET) are recent additions to the treatment options for patients with T2DM. This study assessed the efficacy and safety of therapy with SITA monotherapy and compared the efficacy and safety of SITA/MET with pioglitazone (PIO) monotherapy in patients with T2DM and moderate to severe hyperglycemia.
Materials and methods: After a 2-wk single-blind placebo run-in period 492 eligible patients (18-78 yr of age, HbA_{1c} 7.5-12%, not on an antihyperglycemic agent) were randomized 1:1 to SITA 100 mg once daily (qd) or PIO 15 mg qd (up-titrated to 30 mg after 6 wk). After the initial 12-wk double-blind active treatment period (Phase A) with SITA or PIO monotherapy, patients entered a 28-wk double-blind active treatment period (Phase B). At the beginning of Phase B, patients receiving SITA during Phase A were switched to SITA/MET (up-titrated to 50/1000 mg twice daily over 4 wk) and patients receiving PIO 30 mg qd at the end of Phase A were up-titrated to PIO 45 mg qd at the beginning of Phase B. No inferential testing for between-group differences (SITA vs. PIO) in HbA_{1c} change from baseline was done at the end of Phase A, since maximum glycemic efficacy of PIO was likely not achieved at this time point.
Results: Mean baseline HbA_{1c} was 9.0%. At the end of Phase A, SITA and PIO resulted in significant LS mean changes from baseline in HbA_{1c} (-1.0% SITA, -0.9% PIO), fasting plasma glucose (FPG; -1.5 mmol/L SITA, -1.6 mmol/L PIO) and 2-h post-meal glucose (-2.9 mmol/L SITA, -2.8 mmol/L PIO). At the end of Phase B, the LS mean changes from baseline in HbA_{1c} were -1.8% and -1.4% for SITA/MET and PIO, respectively (between-group difference -0.4%; $p=0.002$). A significantly greater proportion of patients had HbA_{1c} <7% in the SITA/MET group vs. the PIO group (55.0% vs. 40.5%, respectively; $p=0.004$). Significantly larger LS mean reductions at the end of Phase B were

observed with SITA/MET vs. PIO for FPG (-2.6 mmol/L vs. -2.1 mmol/L; p=0.03) and 2-h post-meal glucose (-5.0 mmol/L vs. -3.8 mmol/L; p=0.001). Both SITA/MET and PIO were generally well tolerated. A slightly higher incidence of adverse events (AEs) of abdominal pain (3.2% vs. 0.9%; p=0.083), nausea (2.7% vs. 0.9%; p=0.140), and vomiting (0.9% vs. 0.0%; p=0.150), and a lower incidence of edema (0.9% vs. 6.1%; p=0.003) were observed with SITA/MET vs. PIO. The incidence of hypoglycemia was low and similar in both groups (2.3% and 2.2% with SITA/MET and PIO, respectively). At the end of Phase B, SITA/MET resulted in a LS mean decrease in body weight (-1.1 kg) compared with a LS mean increase (3.4 kg) with PIO (between-group difference -4.5 kg; p<0.001).

Conclusion: In drug-naïve patients, SITA and PIO led to clinically meaningful improvements in glycemic control. Combination therapy with SITA/MET produced a significantly greater improvement in glycemic control compared with PIO. SITA/MET resulted in a decrease in body weight compared with an increase in body weight with PIO. SITA/MET was associated with a significantly lower incidence of edema and a slightly higher incidence of gastrointestinal AEs compared with PIO.

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Safety and efficacy of linagliptin as add-on therapy to a sulphonylurea in inadequately controlled type 2 diabetes

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Background and aims: Linagliptin is an oral dipeptidyl peptidase (DPP-4) inhibitor under development for the treatment of type 2 diabetes (T2D). This 18-week (wk) multi-centre, randomised, double-blind, placebo-controlled parallel group study investigated the efficacy, safety and tolerability of linagliptin administered with sulphonylurea (SU) background therapy in participants with T2D and insufficient glycaemic control.

Materials and methods: Before being randomised to linagliptin (5 mg qd) (n=161), or placebo (n=84), all participants had a 2-wk placebo run-in. Any oral anti-diabetic (OAD) agent other than SU was withdrawn at the beginning of a 4-wk washout period prior to run-in. The SU drug was administered in an unchanged dosage throughout the entire trial duration (including washout and placebo run-in periods). The primary endpoint for the trial was the change in HbA_{1c} from baseline (BL) after 18 wks of treatment evaluated with an analysis of covariance (ANCOVA) adjusted for treatment, prior OAD(s) and BL HbA_{1c}.

Results: Mean BL characteristics (HbA_{1c} 8.6% [SD, 0.8]; fasting plasma glucose (FPG) 179.6 [50.9] mg/dL; age 56.9 [9.9] yrs; BMI 28.3 [5.0] kg/m^2) were similar between the groups. At Wk 18, the adjusted mean change in HbA_{1c} from BL was -0.47%, showing superiority of linagliptin over placebo (p<0.0001). Statistically significant differences between linagliptin and placebo for HbA_{1c} were sustained at all post-BL visits (wks 6, 12 and 18, all p<0.0001). Participants with a BL HbA_{1c} of ≥7.0% were 6 times more likely to achieve a response of HbA_{1c} to ≤7.0% at 18 wks when treated with linagliptin (15.2%) than those receiving placebo (3.7%) (odds ratio (OR) 6.47, p=0.006). The odds of achieving a HbA_{1c} reduction of ≥0.5% at 18 wks was greater for participants treated with linagliptin than in participants treated with placebo (OR 5.12, p<0.0001). At 18 wks, the improvements in glycaemic control were reflected in the difference in adjusted mean change in FPG from BL of -6.4 mg/dL (p=0.24) in favour of linagliptin. The proportion of participants requiring rescue therapy was twice as great for placebo (15.9%) compared to linagliptin (7.6%). Overall, the frequency of reported adverse events (AEs) was similar for the groups (42.2% in the linagliptin group and 42.9% in the placebo group). Of these AEs, 13/161 (8.1%) and 8/84 (9.5%) in the linagliptin and placebo groups, respectively, were considered drug-related by the investigator. AEs of severe intensity were reported for 4 participants (2.5%) in the linagliptin group and none in the placebo group; all other AEs were of mild or moderate intensity. None of the severe AEs was considered as drug-related. Investigator-defined cases of hypoglycaemia occurred in 5.6% and 4.8% of participants in the linagliptin and placebo groups, respectively. No changes in body weight or waist circumference were recorded.

Conclusion: Linagliptin treatment in combination with SU was well tolerated and produced statistically significant and clinically relevant reductions in HbA_{1c}. The safety results were comparable between linagliptin and placebo and, importantly, the addition of linagliptin did not result in a significant increase in hypoglycaemia. Linagliptin may provide an alternative option to uptitrating SU in participants failing on therapy with SU alone or in combination.

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Linagliptin, a novel DPP-4 inhibitor: No need for dose adjustment in patients with renal impairment

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Background and aims: Linagliptin is a potent and highly selective DPP-4 inhibitor in late stage development for the treatment of type 2 diabetes (T2D). Elimination of linagliptin occurs primarily non-renally; this is unique among the currently available DPP-4 inhibitors, which either require dose adjustment or are not recommended in patients with a creatinine clearance (CrCl) of ≤50 mL/min. The purpose of this study was to evaluate the influence of various degrees of renal impairment (RI) on the pharmacokinetics (PK) of linagliptin.

Materials and methods: Linagliptin PK was investigated in subjects with different degrees of RI: mild (CrCl 51-80 mL/min; n=6), moderate (CrCl 31-50 mL/min; n=6), severe (CrCl ≤30 mL/min; n=6), end-stage renal disease (ESRD, n=6) and in healthy volunteers (HV, CrCl >80 mL/min, n=6). In addition, linagliptin PK was compared in 10 patients with T2D with severe RI and in 11 patients with normal renal function (RF). Subjects received 5 mg linagliptin qd as single dose (severe RI and ESRD groups) or for 7 days (HV, mild or moderate RI) or for 10 days (patients with T2D). Plasma and urine concentrations of linagliptin, inhibition of plasma DPP-4, and plasma protein binding were determined. The primary analysis was the comparison of linagliptin exposure in steady state (subjects with mild or moderate RI vs HV and T2D patients with severe RI vs normal RF) or after single-dose (subjects with severe RI/ESRD vs HV).

Results: Steady-state total linagliptin exposure ($AUC_{\tau,ss}$) and maximum concentrations ($C_{max,ss}$) were comparable between subjects with mild RI and the control group, and showed a modest increase of 71 and 42 % in patients with moderate or severe RI, respectively (see table). Under single dose conditions, all patients with RI showed a 30-60% increase in total exposure (AUC_{0-24}) relative to the control groups, regardless of their degree of RI. There was no consistent increase in terminal half-life with deterioration of RF. Accumulation half-lives ranged from 14-15 h in the control groups to 18 h in severe RI. Only a weak correlation was seen between CrCl and steady state exposure or accumulation factor which is further evidence that RI plays a subordinate role in the elimination of linagliptin. Steady state renal excretion of unchanged drug was <7% of the dose in all groups. RI did not alter the plasma protein binding or the correlation between PK and DPP-4 inhibition.

Conclusion: Decreases in renal function had only a minor effect on the elimination of linagliptin. Based on the large safety window of linagliptin, the observed changes in exposure (~40% in severe RI) do not require dose adjustment in patients with T2D and renal impairment when treated with linagliptin.

	Steady-state		Single-dose	
RI Groups	$C_{max,ss}$ [nM]	$AUC_{\tau,ss}$ [nM·h]	C_{max} [nM]	AUC_{0-24} [nM·h]
Mild RI*	0.98 (0.70-1.39)	1.08 (0.91-1.28)	1.26 (0.80-1.96)	1.29 (1.01-1.66)
Moderate RI*	1.46 (0.98-2.19)	1.71 (1.34-2.18)	1.57 (0.77-3.19)	1.56 (1.06-2.32)
T2DM with severe RI#	1.36 (0.97-1.90)	1.42 (1.10-1.82)	1.23 (0.82-1.84)	1.22 (0.92-1.62)
Severe RI*	-	-	1.47 (0.83-2.61)	1.41 (1.04-1.91)
ESRD*	-	-	1.50 (0.94-2.41)	1.54 (1.18-2.00)

*compared with healthy volunteers
#compared with T2D patients with normal RF
Data are geometric mean ratios (90% CI)

Supported by: Boehringer Ingelheim Pharma GmbH & Co. KG

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Linagliptin monotherapy improves glycaemic control in type 2 diabetes patients for whom metformin therapy is inappropriate

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Background and aims: Dose-related adverse events, such as diarrhoea, nausea and abdominal bloating, and a potential risk for lactic acidosis in subjects with renal impairment, may limit metformin use in patients (pts) with type 2 diabetes (T2D). This multi-centre, 18-wk, randomised, double-blind, placebo (PBO)-controlled, parallel group study (followed by an ongoing 34-wk double-blind extension period in which PBO pts were switched to glimepiride) assessed the efficacy, safety and tolerability of the oral DPP-4 inhibitor linagliptin (LI) (5 mg qd) in pts with inadequately controlled T2D for whom metformin therapy is inappropriate due to intolerability or contraindication.
Materials and methods: Hyperglycaemic T2D pts who were treatment-naive (HbA_{1c} ≥7.0 to ≤10.0%, or HbA_{1c} ≥7.0 to ≤9.0% in Canada) or pre-treated with 1 oral anti-diabetes agent (OAD) (HbA_{1c} ≥6.5 to ≤9.0% after a 6-wk washout period) were randomised to LI (n=151) or PBO (n=76) followed a 2-wk PBO run-in (previously treated pts went without medication for 4 wks prior to this). The primary endpoint for the trial was the change in HbA_{1c} from baseline (BL) after 18 wks of treatment evaluated with an analysis of covariance (ANCOVA) adjusted for prior OAD(s), BL HbA_{1c} and reason for metformin ineligibility. This interim analysis was conducted after all pts had completed 18 wks of treatment.
Results: There were no differences between the LI and PBO groups for mean BL characteristics (overall HbA_{1c} 8.09% [SD, 0.93]; fasting plasma glucose (FPG), 10.12 [2.54] mmol/L; age, 56.5 [10.3] yrs; BMI, 29.5 [5.4] kg/m^2). Most of the pts (61.2%) were female. The majority of pts had either normal renal function (55.9%) or mild renal impairment (34.4%). Metformin was inappropriate due to intolerance from gastrointestinal adverse events (AEs) in 93% of the randomised pts, with the remainder of cases due to raised creatinine. After 18 wks of treatment, the adjusted mean difference between LI and PBO was -0.57% with 95% confidence interval (-0.86, -0.29) (p<0.0001) in favour of LI for change in HbA_{1c} (%). Statistically significant differences between LI and PBO for HbA_{1c} were seen by Wk 6 and were sustained through Wk 18. Among pts with BL HbA_{1c} ≥7.0%, 11.8% of pts in the PBO group and 23.5% of the pts in the LI group achieved HbA_{1c} <7.0% at Wk 18. At Wk 18, linagliptin was superior to placebo in reducing the mean FPG from BL (adjusted mean difference -1.14 mmol/L with 95% confidence interval (-31.1, -9.9); p=0.0002). The percentage of pts requiring rescue therapy was higher in the PBO group (17.8%) compared with the LI group (11.6%). The proportion of pts experiencing ≥1 AE classed as drug-related within the LI and PBO groups was 6.6% and 1.3%, respectively. Hypoglycaemia was rare, occurring in 2 pts (1.3%) in the LI group and none in the PBO group and there were no severe cases in either group. No difference in weight was seen between groups.
Conclusion: LI showed superiority, with clinically relevant reduction in HbA_{1c} from BL to Wk 18 compared to PBO. LI was well tolerated, with comparable safety results between LI and PBO. The incidence of hypoglycaemic events during treatment with LI was very low, with no episodes of severe hypoglycaemia, and there was no weight difference between groups. This study shows that LI would be a valuable treatment option for pts with T2D for whom metformin therapy was inappropriate.
Supported by: Boehringer Ingelheim Pharma GmbH & Co. KG

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Diabetes duration and its impact on the effect of dutogliptin, a novel DPP4 inhibitor, on HbA_{1c} and fasting plasma glucose in type 2 diabetes mellitus

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Background and aims: Type 2 diabetes mellitus (T2DM) is characterized by a progressive decline in glycemic control, and disease duration may impact patient therapeutic response to medication. Dutogliptin is a novel and potent dipeptidyl peptidase 4 (DPP4) inhibitor currently in Phase III development for the treatment of T2DM. This post-hoc analysis of a 12-week, multicenter, randomized, double-blind, placebo-controlled trial evaluated the effect of diabetes duration on glycemic response to dutogliptin treatment vs. placebo in patients whose T2DM was not optimally controlled with metformin and/or thiazolidinediones (TZD).
Materials and methods: Patients with HbA1c ≥7.3% currently receiving metformin (≥1500 mg/day or maximally tolerated dose for at least 4 weeks prior to study entry) and/or TZD (at any labeled dose) were stratified by diabetes duration at study entry (< and ≥5 years). Patients were randomized to receive oral doses of dutogliptin 200 mg, 400 mg or placebo once daily. Changes from baseline in HbA1c and fasting plasma glucose (FPG) were assessed after 12 weeks of treatment.
Results: Overall, absolute LS mean reductions from baseline in HbA1c were significantly greater in both the dutogliptin 200 mg (-0.64%) and 400 mg (-0.82%) arms compared to placebo (-0.30%, p<0.01) at week 12. Similarly, the LS mean change from baseline in fasting plasma glucose (FPG, mmol/L) was significantly reduced compared to placebo in the dutogliptin 200 mg (-0.88; p=0.003) and 400 mg (-1.00; p<0.001) groups. However, in patients with <5 years of diabetes duration, reductions in HbA1c and FPG were greater than in patients with ≥5 years of diabetes duration compared to placebo. Patients with diabetes duration <5 years experienced the greatest absolute decrease from baseline in HbA1c in both the dutogliptin 200 mg (-0.778%) and 400 mg (-0.991%) groups compared to placebo (p<0.05). Patients in the dutogliptin 400 mg group with diabetes duration ≥5 years also had statistically significant improvements in both HbA1c (-0.641%) and FPG (-0.976 mmol/L) compared to placebo (p<0.05).
Conclusion: These results demonstrate the positive effects of dutogliptin in early diabetes and support earlier treatment of T2DM with dutogliptin.

	Placebo		*Dutogliptin 200 mg*		*Dutogliptin 400 mg*	
Diabetes Duration	**<5 years**	**≥5 years**	**<5 years**	**≥5 years**	**<5 years**	**≥5 years**
HbA1c						
n	30	53	56	112	69	87
Baseline Mean HbA1c, %	8.439	8.321	8.240	8.559	8.477	8.348
LS Mean Change from Baseline, % (SE)	-0.323 (0.168)	-0.236 (0.127)	-0.778* (0.124)	-0.522 (0.874)	-0.991* (0.111)	-0.641* (0.099)
Fasting Plasma Glucose						
n	31	52	58	109	71	86
Baseline Mean FPG, mmol/L	8.633	9.568	9.273	9.867	9.416	10.128
LS Mean Change from Baseline, mmol/L (SE)	0.254 (0.392)	-0.122 (0.301)	-1.214* (0.285)	-0.697 (0.285)	-1.017* (0.258)	-0.976* (0.235)

*p<0.05 compared to placebo

Supported by: Phenomix Corporation

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Efficacy of saxagliptin in relation to baseline HbA_{1c} in a pooled analysis of 3 add-on pivotal randomised phase 3 clinical trials

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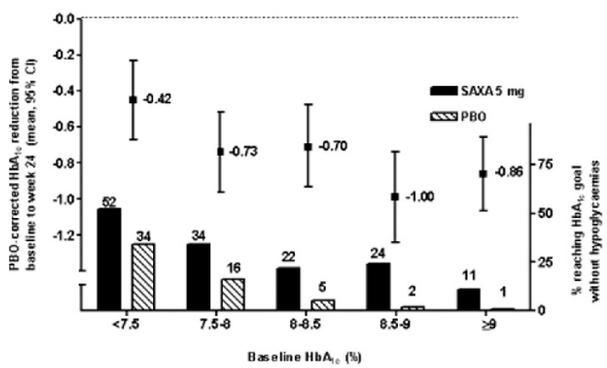
Background and aims: Saxagliptin (SAXA) is a potent, selective dipeptidyl peptidase-4 (DPP-4) inhibitor, approved in Europe for treatment of type 2 diabetes (T2D) in combination with metformin, a sulphonylurea, or a thiazolidinedione (TZD). In the phase 3 programme, SAXA improved control of the glucose triad (HbA_{1c}, PPG and FPG) via a physiological pathway, with weight neutrality and a low incidence of hypoglycaemia. This analysis examines 2 key relationships within the pooled efficacy database with SAXA 5 mg dose, between (1) HbA_{1c} reduction and baseline HbA_{1c} levels, and (2) the proportion of patients reaching HbA_{1c} target (<7.0%) without hypoglycaemia and baseline HbA_{1c} levels, particularly the subgroup of T2D patients slightly above HbA_{1c} target.
Materials and methods: T2D patients (>18y) recruited into the studies, with HbA_{1c} ≥7% and on stable doses of metformin (study CV181-014; NCT00121667), submaximal glibenclamide (CV181-040; NCT00313313), or TZD (CV181-013; NCT00295633). Following a placebo (PBO) run-in period, patients were randomised to SAXA (2.5 or 5 mg od - CV181-040 and CV181-013) or SAXA (2.5, 5 or 10 mg once daily - CV181-014) or (PBO) in addition to ongoing antihyperglycaemic agent. In CV181-040, blinded up-titration of glibenclamide was allowed for patients on PBO. The primary endpoint in all studies was change from baseline HbA_{1c} at week 24. The analysis was carried

out on all patients with a baseline HbA_{1c} value randomised to SAXA 5 mg (n=628) or PBO (n=630) in the 3 studies. Subgroups were defined by baseline HbA_{1c}: <7.5% (SAXA n=126, PBO n=134); ≥7.5–<8% (SAXA n=116, PBO n=135); ≥8–<8.5% (SAXA n=129, PBO n=113); ≥8.5–<9% (SAXA n=102, PBO n=100); ≥9% (SAXA n=155, PBO n=148). Hypoglycaemia was considered to be all symptomatic episodes including confirmed hypoglycaemia (fingerstick glucose <2.8 mmol/L + symptoms). Patients missing data at week 24 were counted as not achieving target HbA_{1c}.

Results: Treatment groups were well balanced for baseline characteristics. SAXA 5 mg od resulted in reduction from baseline to week 24 in mean PBO-corrected HbA_{1c} in all subgroups; greater reductions were seen with increasing HbA_{1c} at baseline (Fig). Percentage of patients reaching target HbA_{1c} (<7.0%) without hypoglycaemia with SAXA 5 mg od and PBO in each subgroup are also shown.

Conclusion: Add-on therapy with SAXA 5 mg od for 24 wks provided clinically relevant reductions in HbA_{1c} vs PBO in patients with inadequately controlled T2D. Pooled subgroup analysis showed that HbA_{1c} reductions were greatest in those with higher baseline HbA_{1c} values. The proportion of patients who achieved target HbA_{1c} without associated hypoglycaemic episodes was highest in those with lower baseline HbA_{1c}. Among patients who were close to target HbA_{1c} at baseline (eg <7.5%), >50% achieved target HbA_{1c} without associated hypoglycaemic episodes over 24 wks, reinforcing the potential role of SAXA as an early add-on therapeutic option.

Mean (95% CI) PBO-corrected change from baseline in HbA_{1c} to week 24 and percentage of patients who achieved target HbA_{1c} without hypoglycaemia for baseline HbA_{1c} subgroups in the SAXA 5 mg od treatment group



Supported by: BMS & AZ

826

Efficacy of saxagliptin according to patient baseline characteristics: a pooled analysis of three add-on pivotal randomised phase 3 clinical trials

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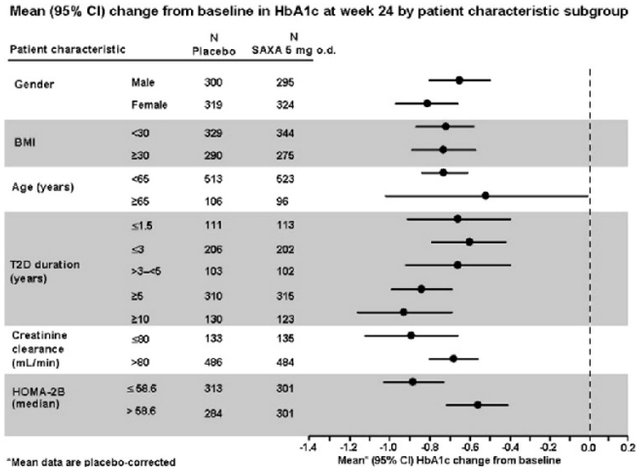
Background and aims: Saxagliptin (SAXA) is a potent, selective dipeptidyl peptidase-4 (DPP-4) inhibitor, approved in Europe for the treatment of type 2 diabetes (T2D) in combination with metformin, a sulphonylurea or a thiazolidinedione (TZD). In phase 3 trials, SAXA demonstrated comprehensive glycaemic control with improvements in all components of the glucose triad (HbA_{1c}, postprandial plasma glucose, fasting plasma glucose) and was associated with weight neutrality and a low incidence of hypoglycaemia. The present analysis, using pooled data from the pivotal phase 3 studies, was conducted to evaluate any potentially important relationships between patient characteristics at baseline and the efficacy of SAXA.

Materials and methods: Patients with T2D recruited into the studies were aged >18 years, HbA_{1c} ≥7%, and were on stable doses of metformin (study CV181-014; NCT00121667), submaximal glibenclamide (CV181-040; NCT00313313), or TZD (CV181-013; NCT00295633). Following a placebo run-in period, patients were randomised to SAXA (2.5 or 5 mg once daily - CV181-040 and CV181-013) or SAXA (2.5, 5 or 10 mg once daily - CV181-014) or placebo in addition to their ongoing antihyperglycaemic agent. In CV181-040, blinded up-titration of the glibenclamide dose was allowed for patients randomised to placebo. The primary endpoint in all studies was change from baseline HbA_{1c} at week 24. This analysis was conducted using placebo-corrected pooled data from all patients randomised to SAXA 5 mg/day in the 3 studies. The following subgroups were defined according to baseline characteristics: gender (male, female); body mass index (BMI; <30, ≥30); age (<65, ≥65 y); T2D duration (≤1.5, ≤3, >3-<5, ≥5, ≥10 y); creatinine clearance (≤80, >80 mL/min,), homeostasis model assessment 2 β-cell function (HOMA-2B; ≤ and >58.6; baseline study median). The efficacy of SAXA (mean change from baseline in HbA_{1c} vs placebo at week 24) was determined for each subgroup category.

Results: Overall, 630 patients were treated with SAXA 5 mg/day in the 3 studies. Baseline characteristics were well balanced across treatment groups within each study (data not shown). Adjusted mean decreases in HbA_{1c} with SAXA 5 mg/day, relative to placebo, were observed for each of the subgroups (figure). Numerically, these mean decreases ranged from -0.52 to -0.93 with relatively larger mean decreases in patients with longer duration of T2D, lower baseline creatinine clearance, and lower baseline HOMA-2B.

Conclusion: This analysis demonstrates that HbA_{1c} lowering from baseline was greater than placebo across diverse demographic and diabetes subgroups over a 24-week period. It supports the use of SAXA 5 mg once daily, as an add-on therapy, in a broad range of patient types.

Mean (95% CI) change from baseline in HbA1c at week 24 by patient characteristic subgroup



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827

Addition of alogliptin vs uptitration of pioglitazone dose in type 2 diabetes mellitus patients on metformin plus pioglitazone therapy

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Background and aims: Maintaining target HbA1c levels in type 2 diabetes mellitus (T2DM) usually necessitates escalation of drug doses and use of combination therapies. In this study, the efficacy of adding alogliptin, an investigational dipeptidyl peptidase 4 inhibitor, was compared with that of increasing the pioglitazone dose in patients experiencing inadequate glycemic control on a regimen of metformin plus pioglitazone.

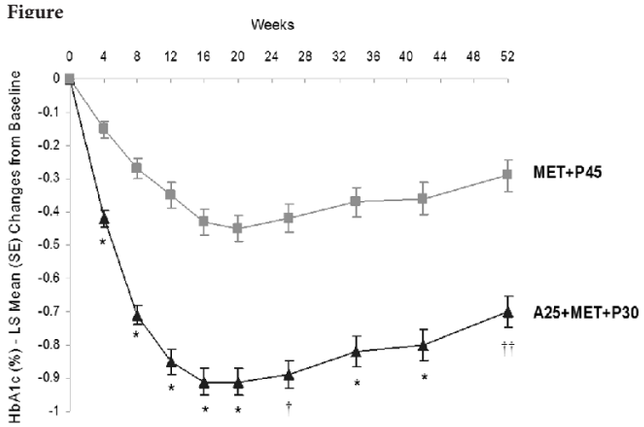
Materials and methods: The addition of alogliptin 25mg (A25) vs the titration of pioglitazone 30 (P30) to 45mg (P45) was evaluated in 803 randomized patients with inadequately controlled T2DM currently treated with metformin (MET; ≥1500mg or maximum tolerated dose) plus P30. The primary endpoint was glycosylated hemoglobin A1c (HbA1c) changes from Baseline (CFB) at Weeks 26 and 52, and the primary analysis involved sequential testing for non-inferiority at Weeks 26 and 52 and superiority at Week 52 only.

Results: The addition of alogliptin (A25+MET+P30) demonstrated superior glycemic control vs the titration of pioglitazone to 45mg (MET+P45), as measured by HbA1c least squares (LS) mean CFB at Week 26 (-0.89% vs -0.42%) and Week 52 (-0.70% vs -0.29%). A25+MET+P30 resulted in significantly (P<0.001) greater HbA1c reductions at all time points (Fig A), regardless of baseline HbA1c (<8%, ≥8%, <9%, ≥9%), and significantly (P<0.001) higher proportions of patients taking A25+MET+P30 vs MET+P45 achieved target HbA1c ≤7.0% (33.2% vs 21.3%) and ≤6.5% (8.7% vs 4.3%) at Week 52. A25+MET+P30 also was significantly more effective than MET+P45 in decreasing FPG (LS mean CFB at Week 52 were -14.6 vs -3.7 mg/dL; P<0.001).

Conclusion: The addition of A25 to an existing T2DM regimen of MET+P30 provided superior glycemic control and potentially improved β-cell function

when compared with MET+P45, with no clinically important differences in safety.

Figure



*P<0.001 vs MET+P45.
†Non-inferior to MET+P45.
††Non-inferior and superior to MET+P45.

Measures of β-cell function significantly (P<0.001) improved with A25+MET+P30 vs MET+P45, as shown by LS mean CFB to Week 52 in pro-insulin/insulin ratio (-0.048 vs -0.007) and HOMA β-cell function (15.02 vs 2.06), while effects on insulin sensitivity were similar (HOMA insulin resistance was 0.35 vs 0.54). Overall, 3.0% of patients reported hypoglycemia.

Supported by: TGRD

828

Efficacy and safety of alogliptin, a potent and highly selective DPP-4 inhibitor, in Japanese patients with type 2 diabetes mellitus

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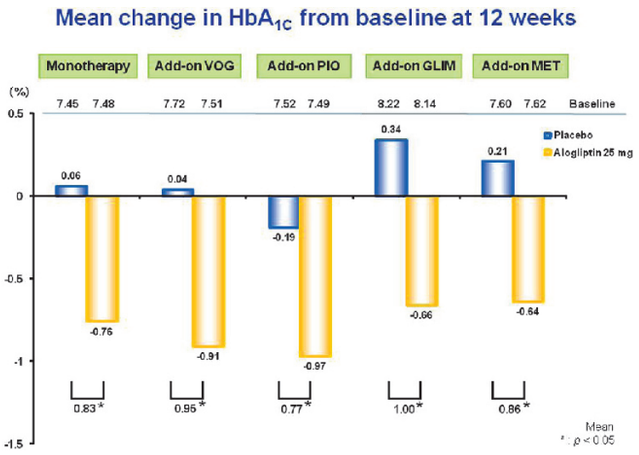
Background and aims: Alogliptin (ALO) is a potent and highly selective DPP-4 inhibitor used for treatment of type 2 diabetes mellitus (T2DM). We investigated the efficacy and safety of ALO used alone and in combination with an α-glucosidase inhibitor (Voglibose : VOG), a thiazolidinedione (Pioglitazone : PIO), a sulfonylurea (Glimepiride : GLIM), or a biguanide (Metformin : MET) in Japanese patients with T2DM.

Materials and methods: Efficacy (decrease in HbA_{1C}, fasting plasma glucose, 2-hr postprandial glucose) and safety (adverse events) of ALO were evaluated after 12-week treatment in five randomized, placebo-controlled, double-blind studies in Japanese patients with T2DM. In addition, long-term efficacy and safety were evaluated in 40-week, open-label extension studies in subjects who had completed the corresponding 12-week study (total treatment duration: 52 weeks). HbA_{1C} was measured by HPLC method according to the Japanese Diabetes Society standard.

Results: A total of 1649 patients were randomized into one of five studies: a monotherapy study (N=480; mean baseline HbA_{1C} 7.53%), a combination study with VOG (N=230; mean baseline HbA_{1C} 7.62%), a combination study with PIO (N=339; mean baseline HbA_{1C} 7.50%), a combination study with GLIM (N=312; mean baseline HbA_{1C} 8.17%), or a combination study with MET (N=288; mean baseline HbA_{1C} 7.57%). At week 12, patients treated with ALO in monotherapy or in combination with VOG, PIO, GLIM, or MET showed statistically significant decreases in HbA_{1C} (Figure), fasting plasma glucose and 2-hr postprandial glucose from baseline compared to placebo. In all 12-week studies, incidences of adverse events, serious adverse events, drug-related adverse events, and hypoglyceamia were similar between ALO and placebo. In the open-label extension studies, ALO as monotherapy or in combination therapies provided substantial improvement in glyceamic control over 52 weeks without weight gain or increased incidence of hypoglyceamia.

Conclusion: Treatment with ALO, whether as monotherapy and in combination with VOG, PIO, GLIM, or MET, significantly improved glyceamic control and was well tolerated in Japanese patients with T2DM.



Supported by: Takeda Pharmaceutical Company Limited

PS 74 GLP-1 analogues: clinical benefits

829

Efficacy and safety of liraglutide compared with glimepiride, both combined with metformin, in an Asian population

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Background and aims: Liraglutide has been investigated for the treatment of type 2 diabetes in Caucasian, African-American and Hispanic populations as part of the LEAD programme. This study aimed to compare liraglutide with glimepiride, both added to metformin, in an Asian population from China, South Korea and India.
Materials and methods: In this 16-week, randomised, double-blind, active controlled trial, 929 patients were randomised to receive either liraglutide (0.6, 1.2 or 1.8 mg once daily; OD) or glimepiride (4 mg OD), both in combination with metformin (1 g twice daily; BD).
Results: Substantial reductions in HbA_{1c} were observed in all treatment groups (Table). Improvements in HbA_{1c} observed with liraglutide 1.2 and 1.8 mg were non-inferior to those reported for glimepiride. Both treatments let to greater improvements in HbA_{1c} in patients previously treated with oral antidiabetic drug (OAD) monotherapy. The proportion of patients reaching HbA_{1c} <7.0% was 36%, 45% and 47% for liraglutide 0.6, 1.2 and 1.8 mg, respectively, compared to 44% for glimepiride. Reductions in fasting plasma glucose were comparable across treatment groups. Liraglutide treatment led to weight loss of 1.8-2.4 kg, while glimepiride treatment resulted in 0.08kg weight gain. Greater reductions in systolic blood pressure (SBP) were observed for liraglutide than glimepiride. Liraglutide was associated with a ~10-fold lower incidence of minor hypoglycaemia than glimepiride. No major hypoglycaemia was reported for liraglutide. The composite endpoint 'HbA_{1c} <7.0%, no weight gain, no hypoglycaemia' was achieved by 29%, 39% and 41% of patients treated with liraglutide 0.6, 1.2 and 1.8 mg, respectively, compared with 17% of patients treated with glimepiride. The most common adverse effects associated with liraglutide were gastrointestinal disorders; however, these were transient in nature and led to very few withdrawals.
Conclusion: These results suggest that treatment with once-daily liraglutide leads to similar improvements in glycaemic control to glimepiride with additional benefits on body weight and SBP and a lower risk of hypoglycaemia. Patients treated with liraglutide are more likely to achieve HbA_{1c} target <7.0% without weight gain and hypoglycaemia. Both liraglutide and glimepiride appear to be efficacious when used early on in patients inadequately controlled with OAD monotherapy. These results from an Asian population are similar to those reported for Caucasian, African-American and Hispanic populations.

	Liraglutide 0.6 mg (n=231)	Liraglutide 1.2 mg (n=233)	Liraglutide 1.8 mg (n=233)	Glimepiride 4 mg (n=231)
Baseline HbA1c, %	8.5 (0.07)	8.6 (0.07)	8.6 (0.07)	8.5 (0.07)
Change in HbA1c, %	-1.14 (0.07)*	-1.36 (0.07)	-1.45 (0.07)	-1.39 (0.07)
% HbA1c <7.0%	35.8*	45.0	46.6	43.9
Baseline FPG, mmol/L	9.8 (0.16)	9.5 (0.14)	9.9 (0.17)	9.6 (0.16)
Change in FPG, mmol/L	-1.7 (0.13)*	-2.1 (0.13)	-2.1 (0.13)	-2.2 (0.13)
Change in weight, kg	-1.80 (0.16)*	-2.35 (0.16)*	-2.44 (0.16)*	0.08 (0.16)
Change in SBP, mmHg	-3.14 (0.82)	-4.25 (0.82)*	-3.54 (0.82)*	-0.91 (0.81)
Minor hypoglycaemia, events/subject year	0.091***	0	0.084***	1.16

Data are mean (SE). ***p<0.0001, *p<0.05 vs glimepiride

Supported by: Novo Nordisk

830

Monotherapy with GLP-1 receptor agonist, Lixisenatide, significantly improves glycaemic control in type 2 diabetic patients

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[1]University of Rochester School of Medicine, Rochester, USA, [2]Tulane University Health Sciences Center, New Orleans, USA, [3]Clínica Cardioprevent, Durango, Mexico, [4]University Hospital Sainte Marguerite, Marseille, France, [5]sanofi-aventis, Antony, France.

Background and aims: This 12-week, randomised, double-blind, placebo-controlled, parallel-group, multicentre, Phase III study assessed the efficacy and safety of lixisenatide (AVE0010; a GLP-1 receptor agonist) as monotherapy in type 2 diabetic patients.
Materials and methods: A total of 361 patients with type 2 diabetes (mean age 53.7 yrs, mean diabetes duration 2.5 yrs) not currently receiving glucose-lowering therapy (HbA_{1c} 7-10%) were randomised to one of four once-daily treatment regimens: lixisenatide 2-step titration (10 µg for 1 week, 15 µg for 1 week then 20 µg; n=120), lixisenatide 1-step titration (10 µg for 2 weeks then 20 µg; n=119), placebo 2-step titration (n=61) or placebo 1-step titration (n=61). A 1-week, single-blind placebo run-in was followed by a 12-week, double-blind, placebo-controlled treatment period. The study was double-blind for active and placebo treatments, and not blinded for drug volume and titration regimens. The primary endpoint was change in HbA_{1c} from baseline to Week 12, analysed using ANCOVA with treatment group, screening strata for HbA_{1c} (<8.0, ≥8.0%) and BMI (<30, ≥30 kg/m²), and country as fixed effects, and baseline HbA_{1c} as covariate. A step-down testing procedure was applied. The two placebo groups were combined in all analyses.
Results: There were significant improvements in HbA_{1c} in both lixisenatide titration groups vs placebo (p<0.0001) (Table). Significantly more patients in the lixisenatide groups achieved HbA_{1c} ≤6.5% (31.9% 2-step, 25.4% 1-step) and <7.0% (52.2% 2-step, 46.5% 1-step) vs placebo (12.5% and 26.8%, respectively; p<0.01). There were also significant improvements in 2-h postprandial and fasting plasma glucose in the lixisenatide groups vs placebo (Table). Mean decreases in body weight were observed in all groups. There was only one (0.4%) serious treatment-emergent adverse event (TEAE) in a lixisenatide-treated patient (2-step group) compared with five (4.1%) in the placebo group. Nine patients discontinued due to a TEAE: five (4.2%) in the lixisenatide 2-step group, three (2.5%) in the lixisenatide 1-step group and one (0.8%) in the placebo group. The most common TEAEs were gastrointestinal: nausea was the most frequent (24.2% for lixisenatide 2-step, 20.2% for lixisenatide 1-step, 4.1% for placebo). Symptomatic hypoglycaemia occurred in three patients (2.5%) in the lixisenatide 2-step group, one (0.8%) in the lixisenatide 1-step group and two (1.6%) in the placebo group, with no cases of severe hypoglycaemia.
Conclusion: Lixisenatide monotherapy administered once daily significantly improved glycaemic control with a pronounced postprandial effect. Lixisenatide monotherapy was safe and well tolerated in patients with type 2 diabetes.

Table. Mean baseline and 12-week changes in glycaemic efficacy variables

			Lixisenatide	
		Placebo (n=121)	2-step titration (n=120)	1-step titration (n=118)
HbA_{1c} (%)	Baseline	8.07 ± 0.92	7.97 ± 0.91	8.06 ± 0.85
	Change from baseline	-0.19 ± 0.12	-0.73 ± 0.12	-0.85 ± 0.12
	LS mean diff. vs placebo	—	-0.54 ± 0.12	-0.66 ± 0.12
	95% CI	—	(-0.79 to -0.30)	(-0.90 to -0.42)
2-h PPG (mmol/L)*	Baseline	13.99 ± 4.78	14.67 ± 3.78	14.55 ± 3.36
	Change from baseline	-0.65 ± 0.56	-4.51 ± 0.57	-5.47 ± 0.55
	LS mean diff. vs placebo	—	-3.86 ± 0.77	-4.82 ± 0.74
	95% CI	—	(-5.38 to -2.35)	(-6.29 to -3.36)
Glucose excursion (mmol/L)*	Baseline	4.72 ± 3.65	5.45 ± 3.02	5.25 ± 2.89
	Change from baseline	-0.67 ± 0.45	-3.77 ± 0.45	-4.36 ± 0.44
	LS mean diff. vs placebo	—	-3.10 ± 0.61	-3.69 ± 0.59
	95% CI	—	(-4.30 to -1.90)	(-4.85 to -2.53)
FPG (mmol/L)	Baseline	8.91 ± 2.17	9.17 ± 1.98	9.02 ± 1.97
	Change from baseline	+0.19 ± 0.26	-0.68 ± 0.25	-0.89 ± 0.25
	LS mean diff. vs placebo	—	-0.87 ± 0.26	-1.08 ± 0.26
	95% CI	—	(-1.37 to -0.36)	(-1.59 to -0.58)

*At selected sites (n=62, 60 and 65 for the placebo, lixisenatide 2-step and lixisenatide 1-step groups, respectively).
Baseline data are mean ± SD. Change from baseline (to Week 12 or LOCF) data are LS mean ± SE.
FPG = fasting plasma glucose; LS = least squares; PPG = postprandial plasma glucose.

Supported by: sanofi-aventis

831

Exenatide significantly improved 24-hour average glucose compared to sitagliptin in patients with type 2 diabetes

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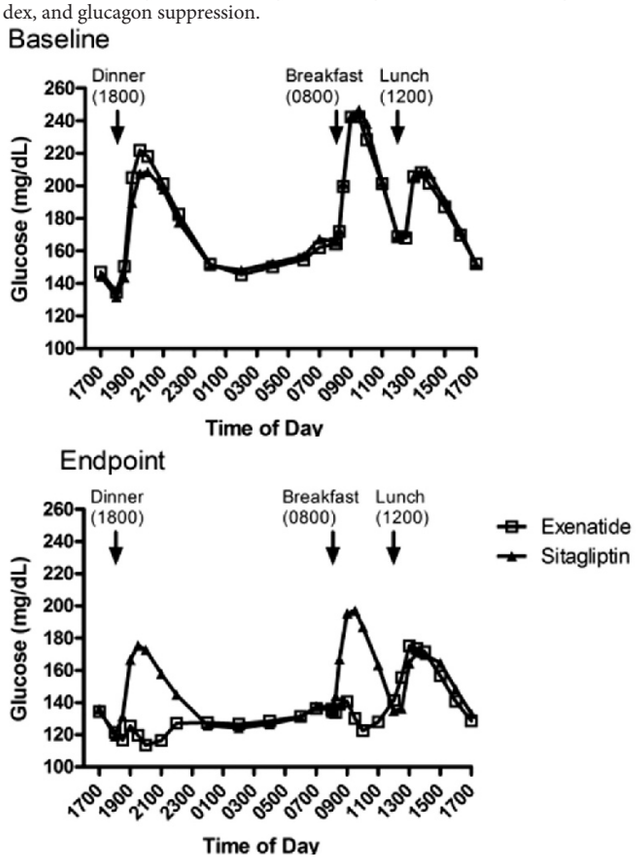
Background and aims: This study compared exenatide, a GLP-1 receptor agonist, and sitagliptin, a DPP-4 inhibitor, with respect to average 24-hour glucose (primary objective) and 2-hour postprandial glucose (PPG), insulin, glucagon, and active GLP-1 (aGLP-1) concentrations and caloric intake in patients with type 2 diabetes.
Materials and methods: This double-blind, double-dummy, randomized crossover study was conducted in 86 metformin or TZD-treated patients: 58% female; BMI 35±5 kg/m^2; A1C 8.3±1.0%. Patients received either exenatide [5µg twice a day (BID) for 1 week, then 10µg BID for 3 weeks] or sitagliptin (100mg one every morning) for 4 weeks. After 4 weeks, each group crossed to the other therapy for 4 weeks. At baseline and the end of each period, patients underwent 24-hour inpatient assessment with hourly or more frequent blood sampling for glucose, insulin, glucagon and aGLP-1. Based on gender and weight, each patient received the same individualized meals across the three 24-hour inpatient periods. 64 patients completed the study.
Results: The figure represents the 24-hour blood glucose profiles for exenatide and sitagliptin at baseline (BL) and endpoint (EP). Both treatments decreased average 24-hour glucose [exenatide 175±40 (BL) to 133±28 (EP) mg/dL; sitagliptin 175±39 (BL) to 146±33 (EP) mg/dL] and 2-hour PPG [exenatide 233±57 (BL) to 122±34 (EP) mg/dL; sitagliptin 233±57 (BL) to 186±51 (EP) mg/dL] from baseline (p<0.001), with the differences favoring exenatide (p<0.001). Both drugs decreased postprandial glucagon and improved the insulinogenic index from BL (p<0.05), but the improvements with exenatide were greater (p<0.001). Sitagliptin increased fasting and postprandial aGLP-1 from BL (p<0.001), while exenatide decreased postprandial aGLP-1 (p<0.05). There was no severe hypoglycaemia during the study and no dropouts due to an adverse event. Adverse events were mild to moderate in intensity and gastrointestinal in nature.
Conclusion: In conclusion, while exenatide and sitagliptin are both incretin-based therapies, exenatide demonstrated significantly better clinical effects compared to sitagliptin on average 24-hour glucose, PPG, insulinogenic index, and glucagon suppression.



Supported by: Eli Lilly and Company and Amylin Pharmaceuticals

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Treatment satisfaction in patients with type 2 diabetes is significantly improved with liraglutide, the once-daily GLP-1 analogue versus sitagliptin, both combined with metformin

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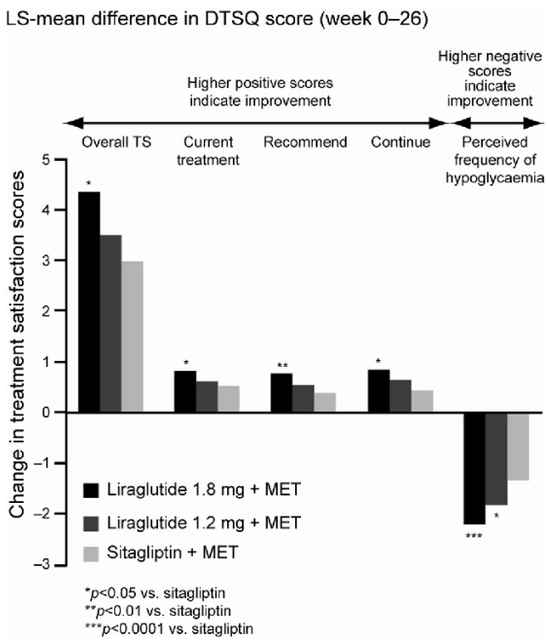
Background and aims: Patient-reported outcomes (PRO) from clinical trials provide information on the impact of a disease on patients' health status, and may identify the extent to which treatments meet patients' needs and expectations. In a 26-week randomised, open-label study (n=665), the injectable once-daily human GLP-1 analogue liraglutide (1.2 or 1.8 mg), led to superior HbA_{1c} reduction vs orally-administered sitagliptin (100 mg OD), both added to metformin (MET; ≥1500 mg daily). Mean HbA_{1c} reduction (%) was 1.5, 1.2 and 0.9 for liraglutide 1.8 mg, 1.2 mg and sitagliptin, respectively (p<0.0001 for both liraglutide doses vs. sitagliptin). Liraglutide patients also lost significantly more weight (~3 kg vs. ~1 kg; p<0.0001). Here we report PRO results from a pre-defined subpopulation of this clinical trial.
Materials and methods: Treatment satisfaction (TS) was assessed using the Diabetes Treatment Satisfaction Questionnaire (DTSQ) at baseline and 26 weeks. Overall TS was calculated by adding satisfaction scores for 'current treatment', 'convenience', 'flexibility', 'understanding', 'recommend', and 'continue'. Higher scores indicate improved TS. For evaluation of perceived frequency of hyper- and hypoglycaemia, higher negative scores indicate improvement. TS scores were analysed by ANCOVA with treatment and country as fixed effects and baseline value as covariate.
Results: 505 subjects were included in the PRO analysis (liraglutide 1.2 mg, n=164; liraglutide 1.8 mg, n=171; sitagliptin, n=170). Baseline characteristics of the PRO subpopulation treatment groups were well balanced. Overall TS was similar between groups at baseline and improved in all groups after 26 weeks. Improvement in overall TS was significantly greater with liraglutide 1.8 mg (4.35) than sitagliptin (2.96) (difference=1.39; 95%CI 0.13; 2.64; p=0.03) (Figure). Patients also reported significantly greater improvement in TS with liraglutide 1.8 mg than sitagliptin on three sub-items: 'current treatment' (difference=0.35; p=0.01), 'recommend' (difference=0.41; p=0.003) and 'continue' (difference=0.44; p=0.01). Patients perceived themselves to be hyperglycaemic significantly less frequently with liraglutide 1.8 mg than sitagliptin (difference=-0.88; p<0.0001, and the same was found with the 1.2 mg dose (difference=-0.49; p=0.01). 'Convenience', 'flexibility', 'understanding' and 'perceived hypoglycaemia' were not statistically different between groups.
Conclusion: Injectable liraglutide leads to greater TS than oral sitagliptin, potentially by facilitating greater improvements in glycaemic control, weight loss and/or perception of superior treatment efficacy.



Supported by: Novo Nordisk

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Exenatide added to a thiazolidinedione with or without metformin resulted in superior glycaemic control versus placebo after 26 weeks of treatment

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Background and aims: Exenatide, thiazolidinediones (TZDs), and metformin regulate blood glucose through unique and potentially complementary mechanisms. The objective of this 26-week study was to investigate the efficacy and safety of exenatide added to a TZD with or without metformin.
Materials and methods: A 26-week, multicountry, randomised, double-blind placebo-controlled study compared exenatide twice-daily vs. placebo, in 165 subjects with type 2 diabetes suboptimally controlled (HbA1c >7.0%) with a TZD or metformin + TZD (mean HbA1c 8.2% [SD 0.9], fasting glucose 9.1 [2.6] mmol/L, weight 93.9 [17.8] kg, diabetes duration 6.4 [4.3] years). After a 2-week, single-blind, placebo lead-in period, subjects were randomly assigned (2:1) to add exenatide or placebo to their current regimens. The primary endpoint was the change in HbA1c from baseline to endpoint (Week 26 or last-observation-carried-forward), using an analysis of covariance model with change in HbA1c from baseline to endpoint as the response variable and treatment, TZD stratum, country, and baseline HbA1c as explanatory variables.
Results: Approximately 95% (157/165) of the subjects were being treated with a TZD and metformin. At endpoint, exenatide reduced HbA1c significantly more than placebo (-0.84% [SE 0.20] vs. -0.10% [0.23], p<0.001; mean treatment difference, -0.74 [95% CI: -1.06% to -0.41%]). Reduction in mean fasting glucose was also significantly greater with exenatide (-0.65 mmol/L [SE 0.46] vs. +0.37 mmol/L [0.52], p=0.009). More subjects achieved HbA1c targets with exenatide (HbA1c ≤7.0%: 49% vs. 37%, p=NS; HbA1c ≤6.5%: 34% vs. 13%, p=0.004). Mean reductions in body weight were -1.4 [SE 0.6] kg with exenatide and -0.8 [0.7] kg with placebo; the between-treatment difference was not significant. Exenatide-treated subjects demonstrated a higher homeostasis model assessment of beta-cell function (HOMA-B index, geometric mean ratio of endpoint to baseline, 1.08 [0.12] vs. 0.84 [0.11]; p=0.009). Change in insulin sensitivity (HOMA-S index) was similar between treatment groups. The most commonly reported adverse events (exenatide vs. placebo) were nausea (12% vs. 2%) and vomiting (8% vs. 0%). Confirmed (blood glucose <3.0 mmol/L) minor hypoglycaemia was experienced by 4% and 2% of subjects treated with exenatide and placebo, respectively.
Conclusion: Adverse events were similar to those previously reported with exenatide with more gastrointestinal symptoms compared to placebo and a low risk of hypoglycaemia. Exenatide added to a TZD, with or without metformin, significantly improved HOMA-B and glycaemic control compared with placebo.
Supported by: Eli Lilly and Company and Amylin Pharmaceuticals, Inc.

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Liraglutide treatment provides greater weight loss with improved glycaemic control than sitagliptin, both combined with metformin

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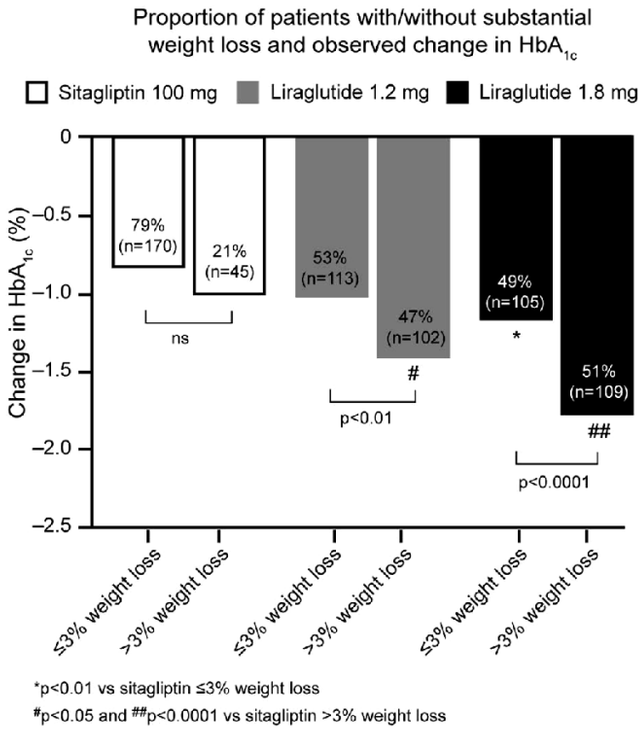
Background and aims: Many standard diabetes treatments are associated with weight gain. Recently, incretin-based therapies have become available for the treatment of type 2 diabetes. In contrast to commonly used diabetes treatments, these incretin-based treatments promote weight loss (GLP-1 agonists) or are weight-neutral (DPP-4 inhibitors). How weight loss relates to improvements in HbA_{1c} remains unclear.
Materials and methods: In a 26-week randomised, open-label study liraglutide (1.2 or 1.8 mg), a once-daily human GLP-1 analogue, was compared with sitagliptin (100 mg), a once-daily DPP-4 inhibitor, both as add-on to metformin, in patients with type 2 diabetes (n=665; baseline HbA_{1c}: 8.5%). This study showed significantly greater reduction in HbA_{1c} (1.2% and 1.5% vs 0.9%, p<0.0001) and weight (2.9 kg and 3.4 kg vs 1.0 kg, p<0.0001) for liraglutide 1.2 mg and 1.8 mg, respectively, than sitagliptin. An ANCOVA analysis using the last observation carried forward (LOCF) intention to treat (ITT) population including effects of treatment, weight and their interaction with baseline HbA_{1c} as a covariate was carried out to investigate the impact of >3% weight reduction on the decrease in HbA_{1c} with liraglutide 1.2 mg (n=215), liraglutide 1.8 mg (n=214) and sitagliptin (n=215).
Results: This analysis demonstrated that treatment with liraglutide 1.2 mg and 1.8 mg led to significantly more patients losing >3% body weight (BW) than sitagliptin (51% [1.8 mg; p<0.0001] vs 21%). Also, weight reductions in the >3% BW change group were significantly greater for liraglutide 1.2 and 1.8 mg than sitagliptin (-5.75 kg and -6.30 kg [p=0.0342], respectively, vs -5.3 kg), while weight changes in the ≤3% BW change group were -0.02 kg, -0.17 kg and +0.38 kg, respectively for liraglutide 1.2 mg, liraglutide 1.8 mg, and sitagliptin. Patients treated with liraglutide who lost >3% weight had significantly greater reductions in HbA_{1c} compared to those who lost ≤3% weight (Figure). In contrast, similar reductions in HbA_{1c} were observed for patients treated with sitagliptin in both weight loss categories. Also, within each weight category, liraglutide treatment led to significantly greater improvements in HbA_{1c} compared with sitagliptin.
Conclusion: Liraglutide treatment resulted in significantly greater reductions in HbA_{1c} in those patients who experienced weight loss of >3%. Importantly, HbA_{1c} reductions were greater following liraglutide treatment in both weight loss categories compared with sitagliptin.



Supported by: Novo Nordisk

835

A meta-analysis of weight loss incurred by liraglutide in patients with type 2 diabetes with and without gastro-intestinal side-effects

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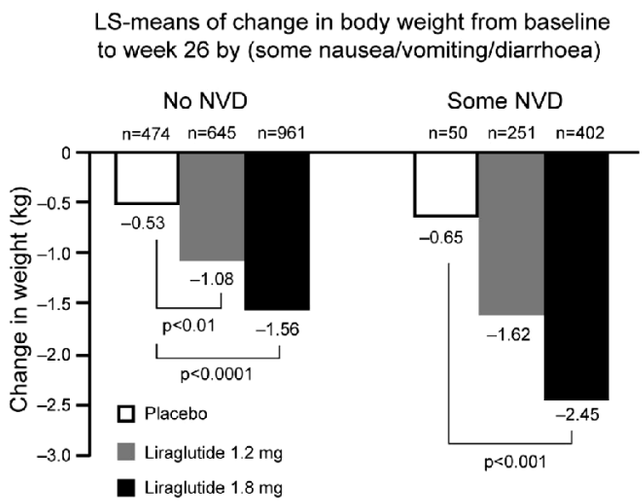
Background and aims: The once-daily human GLP-1 analogue liraglutide is licensed for use as a blood-glucose lowering agent in type 2 diabetes mellitus (T2DM), but also improves cardiovascular risk factors associated with the condition, including systolic blood pressure, and weight. Gastro-intestinal (GI) symptoms such as nausea, vomiting or diarrhoea (NVD) are commonly reported class effects associated with GLP-1 agonists. Clinical studies of liraglutide suggest NVD side-effects are generally experienced early in therapy, and are usually transient and lead to very few withdrawals. Theoretically, NVD could

impact on eating habits and therefore caloric intake; our data explore whether these symptoms alone explain weight loss in patients taking liraglutide.
Materials and methods: This meta-analysis pools data from 6 phase 3 trials conducted in patients (n=2783) with T2DM treated with once-daily liraglutide or placebo for 26 weeks. Our aim was to explore the relationship between weight reduction and the occurrence of NVD. Changes in weight (kg) from baseline were analysed in patients with or without NVD in the different treatment arms using ANCOVA. The analysis included randomised treatment effect, previous anti-diabetes treatment effect, interaction between treatment and NVD, and correction for baseline body weight as covariates.
Results: Weight decrease from baseline to 26 weeks was significant for all groups, except those patients without NVD who were randomised to placebo, and numerical, but non-significant differences in weight loss were seen for the individual liraglutide doses (1.2 vs 1.8 mg) (Figure). The majority of patients taking liraglutide lost weight without NVD (75%). NVD symptoms were reported by 29%, 28% and 10% of patients treated with 1.8 mg liraglutide, 1.2 mg liraglutide and placebo, respectively. Symptoms were generally transient, whereas the weight-loss effect was sustained throughout the studies. No overall interaction was found between the effect of treatment and presence of NVD (p=0.26); however, patients with NVD lost more weight than those without: (p<0.0001 and p=0.0365) for liraglutide 1.8 mg and 1.2 mg, respectively.
Conclusion: Over 2/3 of patients included in the meta-analysis lost significant amounts of weight with liraglutide without experiencing nausea, vomiting or diarrhoea. Weight loss occurs regardless of NVD but is greater in those patients with NVD then in those without NVD.



Supported by: Novo Nordisk

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Meta-analysis of the efficacy of GLP-1R agonists and DPP-4 inhibitors for treatment of type 2 diabetes mellitus

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Background and aims: The number of incretin-based therapies (glucagon-like peptide 1 receptor [GLP-1R] agonists and dipeptidyl peptidase 4 [DPP-4] inhibitors) and the amount of clinical data on their efficacy for treatment of type 2 diabetes mellitus (T2DM) are rapidly increasing. The aim of this meta-analysis was to summarize changes in HbA_{1c}, fasting glucose (FG), and weight for approved or late-stage GLP-1R agonists or DPP-4 inhibitors in studies published or presented at major scientific meetings before Dec 31, 2009.
Materials and methods: Medline, Embase, Biosis, and 2009 ADA and EASD abstract databases were searched for multiple terms including GLP-1, DPP-4, and individual drug names (exenatide [weekly-Ex QW; twice daily-Ex BID], liraglutide, alogliptin, saxagliptin, sitagliptin, vildagliptin). A database of search results was assessed by independent reviewers. Random effects meta-analysis models of the final studies for each therapy examined HbA_{1c}, FG, and weight data.
Results: Reviewers identified 219 unique clinical studies in patients with T2DM. Of these, 63 were randomized controlled clinical trials of 12 to 52 weeks duration with the primary endpoint of change from baseline in HbA_{1c}. The highest maintenance doses of GLP-1R agonists reduced HbA_{1c} to a greater extent than the highest maintenance doses of DPP-4 inhibitors. Ex QW and liraglutide treatment resulted in the greatest mean reductions in FG, whereas lesser mean reductions in FG were observed for Ex BID and DPP-4 inhibitors. Mean weight loss (>2.0 kg) was observed with GLP-1R agonists but not DPP-4 inhibitors. Limitations of the analysis include high inter-trial variation due to differences in amount of data, computation, blinding, comparators, and background therapy.

	GLP-1R Agonists		DPP-4 Inhibitors				
	Exenatide 2 mg QW	Exenatide 10 mcg BID	Liraglutide 1.8 mg QD	Alogliptin 25 mg QD	Saxagliptin 5 mg QD	Sitagliptin 100 mg QD	Vildagliptin 100 mg/d
Studies included	3	12	6	5	4	13	17
Subjects, N	323	2216	1345	902	581	2689	5210
Baseline HbA_{1c}, %	8.5	8.4	8.4	8.1	8.2	8.2	8.3
Δ HbA_{1c}, %	-1.7	-1.0	-1.2	-0.7	-0.7	-0.7	-0.9
[95% CI]	[-2.0, -1.4]	[-1.2, -0.9]	[-1.3, -1.1]	[-0.9, -0.5]	[-0.8, -0.5]	[-0.7, -0.6]	[-1.0, -0.8]
Δ FG, mmol/L[95% CI]	-2.0 [-2.4, -1.6]	-1.1 [-1.3, -0.9]	-1.7 [-2.0, -1.4]	-1.0 [-1.3, -0.7]	-0.9 [-1.3, -0.5]	-0.9 [-1.0, -0.7]	-1.1 [-1.2, -1.0]
Δ Weight, kg	-3.2	-2.1	-2.1	-0.3	-0.4	-0.1	0.0
[95% CI]	[-3.9, -2.4]	[-2.6, -1.7]	[-2.9, -1.2]	[-0.9, 0.3]	[-1.0, 0.1]	[-0.5, 0.3]	[-0.3, 0.3]

Number of studies and subjects are for the primary HbA_{1c} analysis. All Δs are from baseline.

Conclusion: All incretin therapies significantly reduced HbA_{1c} and FG from baseline. Treatment with GLP-1R agonists appeared to be associated with greater reductions in HbA_{1c}, FG, and body weight than were achieved with DPP-4 inhibitors. Further investigation of differences in efficacy between the GLP-1R agonists is warranted.
Supported by: Amylin Pharmaceuticals, Inc. and Eli Lilly & Co.

837

Risk of cardiovascular events in patients with type 2 diabetes treated with exenatide or other glucose-lowering therapies: a retrospective analysis of the LifeLink™ database

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Background and aims: Studies of agents that reduce hyperglycemia in patients with type 2 diabetes have shown differing effects on cardiovascular (CV) outcomes. Glucose-lowering therapies have been associated with increased risk, decreased risk, or neutral risk for CV events and/or mortality. The primary study objective was to assess the relative incidence rate of first CV events among patients with type 2 diabetes prescribed exenatide BID, a GLP-1 receptor agonist, compared to patients treated with glucose-lowering agent(s) other than exenatide (non-Ex).
Materials and methods: Analyses utilized the LifeLink™ database and included patients initiating a new prescription (Rx) for a glucose-lowering agent between June 1, 2005, and March 31, 2009, without Rx for the same agent in the prior 9 months. Patients were initially assigned to the exenatide or non-Ex group based on first new Rx and reassigned if exenatide was prescribed or discontinued. Patients were followed until one of the following occurred: CV event (acute myocardial infarction, stroke, or coronary revascularization procedure), insurance disenrollment, or study end. Patient outcomes adjusted for differences in clinical and demographic characteristics were compared using propensity-score-weighted discrete time survival analysis with time-varying exposure. An ITT analysis of hospitalization was also conducted.
Results: During the study, 39,275 and 381,218 patients were exposed to exenatide and non-Ex therapies, respectively. Age was similar_exenatide: 53 (±9) years; non-Ex: 53 (±11) years; 43.8% of exenatide patients were male and 51.5% of non-Ex patients were male. Exenatide patients were more likely than non-Ex patients to have hyperlipidemia (66.3% vs. 51.7%) and hypertension (65.4% vs. 56.3%). Exenatide-treated patients were 20% less likely to have a CV event than non-Ex patients (HR = 0.80; CI, 0.67-0.95). The exenatide group also had significantly lower rates of CV-related hospitalization (HR = 0.85; CI, 0.76-0.95; P = 0.005) and all-cause hospitalization (HR = 0.95; CI, 0.92-0.99; P = 0.004) than the non-Ex group.
Conclusion: In this analysis, exenatide treatment was associated with a lower risk of CV-related events than treatment with other classes of glucose-lowering therapies.

PS 75 Long acting GLP-1 agonists

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Taspoglutide, a once-weekly human GLP-1 analogue, is superior to Sitagliptin in improving glycaemic control and achieving weight loss in patients with type 2 diabetes: the T-emerge 4 Trial

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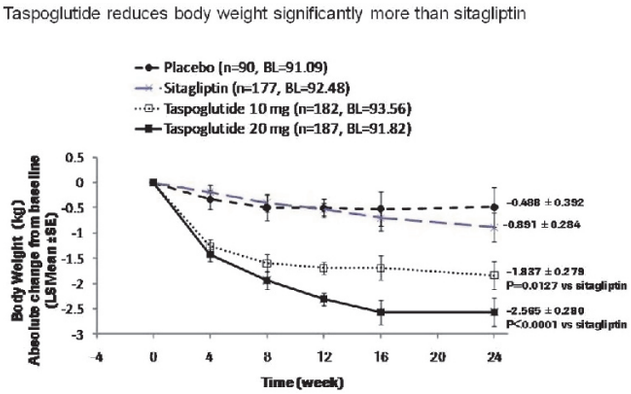
Background and aims: This trial compared taspoglutide, a once-weekly human GLP-1 analog undergoing Phase 3 clinical trials, with sitagliptin and placebo in adult patients (pts) with type 2 diabetes (T2DM) inadequately controlled on metformin.
Materials and methods: In this double-blinded, 24-wk study, 666 adult pts with an A1c between 7%-10% were randomized (2:2:2:1) to subcutaneous taspoglutide 10 mg weekly (Taspo10), taspoglutide 20 mg weekly (Taspo20; titrated after 4 wks of 10 mg), sitagliptin 100 mg QD orally (SIT), or placebo (PL). The primary endpoint was change from baseline in A1c at week 24 using the intent-to-treat population. Superiority of Taspo10 and Taspo20 with PL and non-inferiority (NI) with SIT were tested. If NI with sitagliptin was shown (using 2 sided 95% CI difference and NI margin of ≤0.3), then superiority was tested under closed test procedure.
Results: Baseline characteristics were similar across the groups; average age was 56 yrs, A1c 8.0%, body mass index 32 kg/m^2, and duration of T2DM 6 yrs. Taspo10 and Taspo20, relative to SIT and PL, significantly reduced LS mean (SE) A1c (-1.23% [0.06], -1.30% [0.06], -0.89% [0.06], and -0.10% [0.08], respectively), body weight (Fig), and LS mean (SE) FPG (-2.16 [0.14], -2.33 [0.14], -1.35 [0.14], and -0.07 [0.20] mmol/l, respectively). Both Taspo10 and Taspo20 were superior to SIT for all 3 parameters. Target A1c of ≤7% was achieved by 68%, 72%, 55%, and 18% of pts receiving Taspo10, Taspo20, SIT, and PL, respectively. Gastrointestinal (GI) complaints were the most frequent adverse events and were reported in more pts receiving Taspo than SIT. GI events with Taspo were mostly mild and moderate in severity and transient in most pts; the withdrawal rate was 15%, 10%, 0.5%, and 1% of pts on Taspo10, Taspo20, SIT, and PL, respectively.
Conclusion: Once-weekly Taspo provided superior glycemic control and weight loss compared with SIT, while SIT had a lower incidence of GI adverse events. (NCT00754988)



Supported by: Roche

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Taspoglutide, a once-weekly human GLP-1 analog, as monotherapy significantly lowers HbA$_{1c}$ and body weight in patients with type 2 diabetes: results from the T-emerge 1 phase 3 trial

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Background and aims: Taspoglutide is a once-weekly human GLP-1 analog in Phase 3 clinical trials for T2D. This was a randomized, double-blind, placebo-controlled trial of taspoglutide monotherapy in drug-naïve patients.
Materials and methods: Adults (n=373) uncontrolled on diet and exercise with HbA1c between ≥6.5% and ≤10% were randomized 1:1:1 to subcutaneous taspoglutide 10 mg weekly (Taspo10), taspoglutide 20 mg weekly (Taspo20; after 4 weeks of Taspo10), or placebo (PL) for 24 weeks. The primary endpoint was the absolute change from baseline in HbA1c (%) after 24 weeks.
Results: Patient demographics were similar among the 3 groups; ~63% were women, mean age 54 yrs, body mass index 32 kg/m^2, mean HbA1c was 7.6% and duration of T2D ~2 yrs. Primary efficacy results at week 24 in the intent-to-treat population (ITT), using last observation carried forward (LOCF) are shown in the Table. Reductions from baseline in HbA1c and FPG were significantly greater with Taspo10 and Taspo20 than with PL. Target HbA1c of ≤6.5% was achieved by 59.8% (95% confidence interval [CI], 50.1-69.0), 66.1% (95 % CI, 57.2-74.3), and 17.4% (95% CI, 11.0-25.6) in the Taspo10, Taspo20, and PL groups, respectively. Reduction in body weight in the Taspo20 group was significantly greater than in PL (Table). The most frequently reported adverse events were nausea and vomiting, occurring at a greater incidence in the Taspo10 and Taspo20 groups than PL. Withdrawals due to gastrointestinal adverse events occurred in 5.2%, 7.8% and 0.8% of patients in the Taspo10, Taspo20, and PL groups, respectively.
Conclusion: Once-weekly taspoglutide as monotherapy in drug-naive patients with low baseline HbA1c, significantly improved glycemic control, reduced body weight, and was well tolerated. (NCT00744926)

LSMean ± SE	Taspo10 (n=112)	Taspo20 (n=127)	PL (n=115)
Baseline HbA1c (%)	7.52±0.10	7.66±0.09	7.62±0.09
Change from baseline HbA1c (95% Confidence Interval [CI])	-1.01±0.07 (-1.14, -0.88)	-1.18±0.06 (-1.31, -1.06)	-0.09±0.07 (-0.22, 0.04)
Diff from PL	-0.92±0.09**	-1.09±0.09**	_
Baseline FPG (mmol/l)	8.75±0.24	8.97±0.22	8.64±0.23
Change from baseline FPG (95% CI)	-1.55±0.17 (-1.89, -1.21)	-1.90±0.17 (-2.23, -1.58)	-0.08±0.17 (-0.42, 0.25)
Difference from PL	-1.47±0.24**	-1.82±0.23**	_
Baseline body weight (kg)	88.40±1.70	84.97±1.60	87.40±1.68
Change from baseline body weight (95% CI)	-1.45±0.32 (-2.07, -0.84)	-2.25±0.30 (-2.84, -1.65)	-1.23±0.31 (-1.84, -0.62)
Difference from PL	-0.22±0.43	-1.01±0.42*	_

*P<0.05; **P<0.0001 vs PL

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Taspoglutide, once-weekly human GLP-1 analogue, compared to BID Exenatide improves glucose tolerance and insulin secretion in patients with type 2 diabetes: T-emerge 2 meal tolerance test

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Background and aims: T-emerge 2 was a randomized, open-label, 24-week trial comparing subcutaneous taspoglutide 10 mg weekly (Taspo10), taspoglutide 20 mg weekly (Taspo20; titrated after 4 wks of Taspo10), with exenatide 10 mcg BID (Exe; after 4 weeks of Exe 5 mcg) in patients inadequately controlled on metformin, a thiazolidinedione, or both. T-emerge 2 demon-

strated that once-weekly Taspo provided better glycemic control than Exe. This report focuses on a subset of T-emerge 2 participants undergoing a standardized meal comparing Taspo to Exe which has been previously shown to lower postprandial glucose.
Materials and methods: Meal tolerance tests (MTT) were performed at baseline and at week 24 in a subset of Taspo 10, Taspo 20 and Exe patients (n=42, 39, and 67, respectively). Blood samples for glucose, insulin, glucagon, and C-peptide were obtained before and after (30, 60, 90, 120, and 180 min) ingestion of a standardized liquid meal.
Results: The 2-h postprandial, $mean_{0\text{-}3h}$ and $AUC_{0\text{-}3h}$ glucose during the MTT was reduced to a similar extent in all groups and the time profile of the postprandial glucose showed a similar pattern. Taspo10 and Taspo20 significantly increased insulin from baseline (both mean and $AUC_{0\text{-}3h}$) while the increase in insulin from baseline was not significant for Exe (Table). Although changes from baseline in C-peptide were not significant within any treatment group, the mean change from baseline (both $mean_{0\text{-}3h}$ and $AUC_{0\text{-}3h}$) was significantly increased in Taspo10 vs. Exe. Mean glucagon showed significant decreases in all groups (Table).
Conclusion: Taspoglutide and Exe improved glucose tolerance and reduced glucagon responses to a similar extent while taspoglutide alone significantly improved insulin secretion from baseline in patients with T2DM. (NCT00717457)

LSMean ± SE	Taspo10	Taspo20	Exe
BL glucose $AUC_{(0\text{-}3hr)}$ (mmol*h/L)	35.91±1.17	39.05±1.21	37.47±0.92
Percent change from BL glucose $AUC_{(0\text{-}3h)}$ (95% CI)	-24.97±3.60 (-32.09, -17.85)	-27.05±4.16 (-35.27, -18.83)	-24.50±3.62 (-31.65, -17.35)
BL insulin $AUC_{(0\text{-}3hr)}$ (uU*h/mL)	77.99±8.40	110.40±8.40	77.87±6.93
Percent change from BL insulin $AUC_{(0\text{-}3h)}$ (95% CI)	53.80±15.44 (23.09, 84.52)	47.23±20.07 (7.32, 87.14)	26.36±18.33 (-10.09, 62.81)
BL $glucagon_{(0\text{-}3hr)}$ (pmol/L)	26.58±1.20	26.35±1.25	25.23±0.93
Percent change from BL glucagon (95% CI)	-2.33±1.08 (-4.46, -0.20)	-2.64±1.22 (-5.05, -0.22)	-2.17±1.06 (-4.26, -0.07)
BL C-peptide $AUC_{(0\text{-}3hr)}$ pmol*h/L	5025	5229	4936
Percent change from BL C-peptide (95% CI)	9.49±6.05* (-2.49, 21.46)	-2.24±7.02 (-16.14, 11.66)	-4.54±6.05 (-16.52, 7.45)

BL, baseline; CI, confidence interval*P<0.05 vs Exe

Supported by: Roche

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Disease progression modelling to quantify the effects of exenatide twice daily and once weekly formulations on HbA_{1c} in patients with type 2 diabetes
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Exenatide, a GLP-1 receptor agonist, is given twice daily (Ex BID) and indicated for patients with type 2 diabetes; and a once weekly (Ex QW) formulation is under development. The purpose of this analysis was to implement a population disease progression pharmacodynamic modeling approach to quantify the effects of demographic, clinical, and formulation characteristics on HbA_{1c} and to examine the effects of exenatide exposure on HbA_{1c} when transitioning patients (PT) from the Ex BID formulation to Ex QW. Clinical data was pooled from: (a) 3 Ex BID Phase 3 trials in PT treated with metformin (MET) and/or a sulphonylurea (SU); (b) 1 Ex QW Phase 2 trial in PT treated with MET and/or Diet and Exercise (DE); and (c) 1 comparative Phase 3 trial where PT, treated with MET, SU, thiazolidinedione (TZD), and/or DE, were initially randomised to Ex BID or Ex QW. Patients on Ex BID transitioned to Ex QW. The data set included HbA_{1c} measurements from 1272 exenatide and 496 placebo PT for up to 52 wk. A disease progression model was constructed using nonlinear mixed effects modeling for repeated measures data to describe the natural HbA_{1c} time course, placebo effects, exenatide effects, and the effects of patient and formulation characteristics on HbA_{1c}. The placebo and drug effects on HbA_{1c} were calculated from the estimated model parameters. The estimated baseline HbA_{1c} was 8.4% for the typical patient (100 kg, male, 55 y, no concomitant MET, TZD, or SU therapy). No significant differences in baseline HbA_{1c} were detected for gender or for concomitant administration with MET or SU. The baseline HbA_{1c} value was reduced by 0.44% (0.12, 0.69) for PT administered TZD. The placebo effect translated to a mean absolute reduction of 0.3% at 8 wk, and returned to baseline by approximately 30 wk. Mean (95% CI) steady-state HbA_{1c} levels were reduced by 0.8% (0.7, 0.9) and 1.2% (1.0, 1.5) for 5 μg and 10 μg Ex BID, respectively, and reduced by 1.4% (0.7, 2.8) for a 2 mg Ex QW dose. Patients who transitioned from 10 μg Ex BID to 2 mg Ex QW demonstrated an additional 0.2% response to Ex QW administration, demonstrating a clinical advantage of prolonged exenatide exposure.
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DURATION-2: effect of switching to once-weekly exenatide from maximum daily doses of sitagliptin or pioglitazone
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Background and aims: In the 26-week, double-blind, double-dummy assessment period of the DURATION-2 trial in patients with type 2 diabetes on metformin, treatment with the once-weekly GLP-1 receptor agonist exenatide (Ex QW) resulted in greater improvements in glycaemic control and weight compared to maximum approved doses of sitagliptin and pioglitazone. In the subsequent 26-week, open-label, uncontrolled assessment period, randomised oral medications were discontinued and all patients received Ex QW. The safety and efficacy of Ex QW treatment for 52 weeks, as well as the effects of switching from sitagliptin or pioglitazone to Ex QW, were evaluated.
Materials and methods: Of the 364 patients who continued into the open-label period and received at least 1 Ex QW dose (study entry baseline: HbA_{1c} 8.5±1.1%, fasting plasma glucose [FPG] 9.0±2.5 mmol/L, weight 88±20 kg), 319 patients (88%) completed 52 weeks. Results are reported for the 52-week evaluable population.
Results: Patients who received Ex QW throughout the trial demonstrated significant improvements from baseline in HbA_{1c}, FPG, and weight. At the end of the 52-week assessment period, 39% of patients treated with Ex QW for 52 weeks achieved HbA_{1c} ≤6.5% and 62% achieved the FPG target ≤7.0 mmol/L. Patients who switched from sitagliptin to Ex QW demonstrated significant incremental improvements in HbA_{1c}, FPG, and weight, and significantly more patients achieved HbA_{1c} ≤6.5% (week 26: 18% → week 52: 36%; P<0.001) and FPG ≤7 mmol/L (week 26: 38% → week 52: 58%; P<0.001). Patients who switched from pioglitazone maintained the improvements in HbA_{1c} and FPG observed during the initial 26 weeks, and a similar proportion of patients achieved HbA_{1c} ≤6.5% (40%) and FPG ≤7 mmol/L (59%) compared to patients treated with Ex QW for 52 weeks. However, switching from pioglitazone to Ex QW resulted in a significant weight reduction that reversed the weight gain associated with 26 weeks of pioglitazone treatment. Improvements in systolic blood pressure (SBP) with Ex QW and pioglitazone at week 26 were maintained at week 52 (-2.9±1.3 mmHg and -2.2±1.3 mmHg, respectively), while SBP was reduced by -2.7±1.1 mmHg (P<0.05) in patients who switched from sitagliptin to Ex QW (week 52: -2.9±1.2 mmHg). Ex QW was generally well tolerated and adverse events were predominantly mild or moderate in severity. Treatment-emergent nausea was the most frequent adverse event in this assessment period (ITT: Ex QW-only: 5%; sitagliptin → Ex QW: 11%; pioglitazone → Ex QW: 10%). No major hypoglycaemia was observed.
Conclusion: Switching to once-weekly exenatide from daily sitagliptin or pioglitazone resulted in improved or sustained glycaemic control with weight loss.

	HbA_{1c} (%)	FPG (mmol/L)	Body Weight (kg)
EQW-only (week 0-52)			
Δ from baseline at week 52	-1.6±0.1	-1.8±0.3	-1.8±0.5
Sitagliptin (week 0-26) → EQW (week 26-52)			
Δ from week 26 to week 52	-0.3±0.1*	-0.7±0.2*	-1.1±0.3*
Δ from baseline at week 52	-1.4±0.1	-1.7±0.3	-2.0±0.5
Pioglitazone (week 0-26) → EQW (week 26-52)			
Δ from week 26 to week 52	-0.1±0.1	0.0±0.2	-3.0±0.3*
Δ from baseline at week 52	-1.6±0.1	-1.7±0.3	+0.4±0.5

LS mean ± SE; *P<0.05 vs. week 26

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DURATION-5: Exenatide once weekly resulted in significantly greater improvement in glycaemic control than exenatide twice daily in patients with type 2 diabetes

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Background and aims: Treatment with the GLP-1 receptor agonist exenatide results in improved glycaemic control and weight loss in patients with type 2 diabetes. This 24-week, randomised, open-label, comparator-controlled study compared treatment with exenatide once weekly (Ex QW; 2 mg) to exenatide twice daily (Ex BID; 10 mcg).
Materials and methods: The study was conducted in 252 intent-to-treat patients with type 2 diabetes (baseline [mean±SD]: HbA_{1c} 8.4±1.2%, fasting plasma glucose [FPG] 9.5±2.6 mmol/L, weight 96±20 kg). Patients were drug-naïve (18.7%) or treated with one (46.8%) or a combination (34.5%) of oral antidiabetic medications.
Results: Over 24 weeks of therapy, Ex QW resulted in significantly greater decreases from baseline (LS mean±SE) versus Ex BID in HbA_{1c} (-1.6±0.1% [Ex QW] versus -0.9±0.1% [Ex BID]; P<0.0001) and FPG (-1.9±0.3 mmol/L [Ex QW] versus -0.7±0.3 mmol/L [Ex BID]; adjusted P=0.0008). Improvements in HbA_{1c} were consistently observed across different background antidiabetic therapies. A significantly greater percentage of Ex QW patients (58.1%) achieved the HbA_{1c} target of <7% compared to Ex BID patients (30.1%; adjusted P<0.0001). A total of 41.1% of Ex QW patients achieved the HbA_{1c} target of ≤6.5% compared to 16.3% Ex BID patients (P<0.0001). Progressive reductions in mean body weight were observed in both treatment groups (change from baseline to Week 24 (LS mean±SE): -2.3±0.4 kg [Ex QW]; -1.4±0.4 kg [Ex BID]; not significant). A total of 71% of Ex QW patients and 51% of Ex BID patients had improvements in both body weight and HbA_{1c} after 24 weeks of therapy. Reductions in sitting systolic blood pressure from baseline to Week 24 were observed with Ex QW (LS mean [95% CI]: -2.9 [-5.2,-0.7] mm Hg) and Ex BID (-1.2 [-3.5, 1.2] mm Hg). Ex QW and Ex BID were well tolerated. Nausea, the most frequent adverse event, occurred less frequently with Ex QW (14%) than with Ex BID (35%) and was predominantly transient and mild or moderate in intensity. Injection-site reactions were infrequent and generally mild in intensity, but occurred more often with Ex QW compared to Ex BID. No major hypoglycaemia occurred. Minor hypoglycaemia was infrequent and occurred only in patients using a concomitant sulphonylurea. No change in mean calcitonin concentrations was observed during the study. Pancreatic-amylase and lipase concentrations were variable, both pre- and postbaseline, and changes in these enzymes were not predictive of gastrointestinal adverse events.
Conclusion: Continuous exenatide exposure via Ex QW therapy resulted in superior glycaemic control with fewer gastrointestinal adverse events compared to Ex BID in patients with type 2 diabetes. Both groups lost weight.

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VRS-859, a monthly dosed glucagon-like peptide-1 (GLP-1) analogue, provides long-term glucose control in mouse models and lacks toxicity in mice and monkeys

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Background and aims: VRS-859 is a novel, monthly dosed glucagon-like peptide-1 (GLP-1) analogue, being developed for treatment of type 2 diabetes (T2DM). VRS-859 is a fusion protein containing the GLP-1 analogue, exenatide, and a long hydrophilic tail of natural amino acids, XTEN, which increases the half-life. Mouse studies were performed to determine the relationship between the pharmacokinetics (PK) and pharmacodynamics (PD) of VRS-859. Toxicology studies were performed in mice and monkeys to ensure adequate safety for the proposed human dose. Single subcuntaneous doses of VRS-859 are being evaluated in a placebo controlled blinded study of patients with T2DM.
Materials and methods: Normal mice were treated with VRS-859 (1.2 to 120 nmol/kg) or exenatide and assessed for glycemic control and insulin secretion after intraperitoneal glucose tolerance test (IP GTT; AUC_{Glu} measured over 120 min and compared to placebo treatment) performed at intervals up to 72 hrs post-dose. VRS-859 (120 nmol/kg Q2D or 240 nmol/kg Q4D) or exenatide (infusion pump) was administered to diet induced obese (DIO) mice for 28 days. PK and toxicology of VRS-859 were assessed in mice dosed every other day with up to 50 mg/kg VRS-859 and cynomolgus monkeys dosed weekly with up to 35 mg/kg VRS-859 for 28 days. Clinical pathology, cardiovascular safety, and complete histology were performed in the toxicology studies. T2DM patients are being enrolled in a Phase 1 placebo controlled single ascending dose study of VRS-859. Patient evaluations include safety, tolerability, fasting plasma glucose, glycated albumin, HbA1c, oral glucose tolerance tests, insulin, and antibodies to VRS-859.
Results: Up to 48 hrs after IP VRS-859 dosing (120 nmol/kg), mice maintained a significant improvement in glucose tolerance (AUC_{Glu} = -53%), while exenatide only demonstrated significant improvement up to 1 hr post-dose. Insulin secretion after IP GTT was also increased up to 48 hrs after IP VRS-859 (120 nmol/kg) administration. There was a dose dependent glucose tolerance noted at 1 hr after dosing 1.2, 12 or 120 nmol/kg VRS-859 (AUC_{Glu} : -1, -54, and -66%, respectively). Correlating plasma levels of VRS-859 with the PD effects suggested that a human plasma level of 200 ng/mL VRS-859 may be sufficient to ensure glycemic control. After 28 days, DIO mice treated with VRS-859 had a significantly decreased body weight ($p < 0.01$) and fasting blood glucose ($p < 0.05$) compared to placebo, but exenatide infusion did not cause significant reduction in fasting blood glucose. The no observed adverse effect level of VRS-859 was 50 mg/kg in mice and 35 mg/kg in monkeys. Allometric scaling of the VRS-859 pharmacokinetics indicated a projected human terminal half-life of 139 hrs. Therefore, a 100 mg dose of VRS-859 may provide plasma levels sufficient to ensure glycemic control for one month in humans. Preliminary Phase 1 results including the pharmacokinetics and pharmacodynamics as well as safety and tolerability will also be presented.
Conclusion: VRS-859 provides sustained glycemic control and weight loss. A single subcutaneous dose of VRS-859 may enable glycemic control in a T2DM patient for one month.

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Treatment with LY2189265 (GLP-1 analogue) causes larger decreases in postprandial glucose excursion in Hispanics compared to Non-Hispanic Caucasians with uncontrolled type 2 diabetes: an EGO Study exploratory analysis

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Background and aims: The Hispanic population is severely affected by the increasing incidence of type 2 diabetes mellitus, but studies of the differential responses to drug therapy in this group have received little attention. The objective of this exploratory analysis of the EGO study is to examine differences in glycemic control between Hispanic (H) and Non-Hispanic (NH) Caucasian populations in response to treatment with the long-acting glucagon-like peptide-1 (GLP-1) analog LY2189265 (LY).
Materials and methods: Subjects were randomized to once-weekly subcutaneous injections of either placebo or 1 of 3 LY dose regimens: 1) 1.0 mg for 16 weeks; 2) 0.5 mg for 4 weeks then titrated to 1.0 mg for 12 weeks; or 3) 1.0 mg for 4 weeks then titrated to 2.0 mg for 12 weeks. The primary metabolic measure for comparison between the 2 ethnic groups was glycemic control, as measured by HbA1c change from baseline at 16 weeks. Secondary measures were change in 1) fasting blood glucose (FBG); 2) β-cell function (HOMA2%B); and 3) glucose excursion (AUC) response to a solid mixed meal test. Differences between groups were tested using a two-sample T-test and nominal significance level of 0.05 for comparisons.
Results: In all randomized subjects, the H group had a statistically significantly higher baseline HbA1c compared to the NH group (8.4±1.0%, n=88 vs. 8.1±0.9%, n=150, p=0.006). The 177 patients randomized to LY treatment (62 H and 115 NH) had similar baseline characteristics; however, baseline HbA1c was 8.5±1.0% in H (n=62) compared to 8.2±0.9% in NH (n=114), p=0.078, and baseline postprandial AUC glucose excursion was significantly higher in H compared to NH (12.2±3.9 [mmol/L]•hr, n=60 vs. 10.3±5.3 [mmol/L]•hr, n=112, p=0.007). In response to LY treatment, both groups achieved statistically significant reductions in FBG and HbA1c. The H population experienced a greater reduction in HbA1c compared to NH at endpoint (-1.5±1.0%, n=61 vs. -1.1±0.8%, n=111, p=0.020), but changes in FBG were not significantly different between groups (-2.3±2.8 mmol/L, n=55 H vs. -1.8±2.5 mmol/L, n=92 NH, p=0.253). Changes in HOMA2%B were not significantly different between groups, but there was a 5.6-fold greater decrease

in postprandial AUC glucose excursion in the H group compared to the NH group (-2.8±3.8, n=56 vs. -0.5±5.7, n=89, [mmol/L]•hr, p=0.003). The percentage of subjects with ≥1 treatment-emergent adverse event in response to treatment with LY was similar between groups (58.1%, n=36 in H vs. 60%, n=69 in NH, p=0.803).
Conclusion: In conclusion, in response to treatment with LY, reductions in HbA1c were significantly greater in H compared to NH. This greater decrease in HbA1c in the H population appears to be due to the greater reduction in postprandial glucose excursion in H. Further studies are warranted to prospectively evaluate differential effects of LY treatment in the Hispanic population with type 2 diabetes mellitus.

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Normalising action of GLP-1 and Exendin-4 on bone metabolism in obese state

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Background and aims: The bone anabolic action of GLP-1 and exendin-4 (Ex-4) in normal, insulin resistant and type 2 diabetic rats has been demonstrated, the effect of GLP-1 being suggested to take part, at least partially, through specific receptors, distinct in structure and/or function from the pancreatic GLP-1 receptor. Hypercholesterolemia seems to be related with low levels of bone mineral density. Here we have explored the possible *in vivo* effect of GLP-1 and Ex-4 on bone turnover and other markers, in a diet-induced obesity rat model (OB), compared to normal (N).
Materials and methods: OB was obtained in adult Wistar rats by chronic feeding during five weeks with a *cafeteria diet*, consisting in standard chow supplemented with cookies, liver paste, bacon, and whole milk containing sucrose (333 g/l) and 10 g/l of a mineral and vitamin complex (65% energy from lipids). The N group was fed with standard chow and water *ad libitum* (8% energy as fat). Although weight was not different between N and OB rats, the OB model showed higher plasma glucose (78±2 mg/dl, n=12), triglycerides (153±13 mg/dl, n=11) and cholesterol (92±4 mg/dl, n=12) than those in N (overall mean: 37±5% Δ N-rats, $p<0.02$); no significant differences with N were detected in plasma insulin or GLP-1 -by RIA-. OB rats were 3-days continuously treated -osmotic pump- with saline (control), GLP-1 (0.86 nmol/kg/h) or Ex-4 (0.1 nmol/kg/h). In fed conditions, blood samples were taken before (basal) and by the end of the treatment for plasma measurements; then, rats were sacrificed and the femorae, tibiae and L1-L4 vertebrae were collected. In the tibia, after total RNA extraction, osteocalcin (OC), osteoprogeterin (OPG) and RANK ligand (RANKL) gene expression -by RT-PCR- was determined; femoral and lumbar spine bone mineral densities (BMD) were also measured (Lunar Piximus).
Results: In the OB group, GLP-1 lowered the higher than normal triglycerides value (-33±6% Δ OB-basal, $p<0.05$, n=6 rats), without modifying that of cholesterol; however, Ex-4 induced a reduction in both triglycerides (-44±5% Δ OB-basal, $p<0.01$, n=6) and cholesterol (-19±3% Δ OB-basal, $p<0.01$, n=5). While plasma glucose, creatinin and insulin were not apparently different in any group or condition, calcium in OB was slightly higher ($p<0.01$) than that in N, without differences in the phosphates values; GLP-1 induced a decrease in the two latter parameters (overall mean: -8±2% Δ OB-basal, $p<0.01$, n=6) while Ex-4 reduced only the phosphate levels (-17.1±3.2% Δ OB-basal, $p<0.01$, n=6). In OB rats (n=12), BMD in femur (0.144±0.03 g/cm^3) and lumbar spine (0.128±0.03 g/cm^3) was lower (overall mean: -19±3% Δ N, $p<0.001$) than those in N-control rats (n=6-12), and either GLP-1 or Ex-4 exerted a normalizing effect (overall mean: 94±1% N, $p<0.001$). In the tibia of OB rats, OC mRNA level was equal to that in N, and both GLP-1 and Ex-4 induced an increase (overall mean: 2.23±0.21 times OB-control, $p<0.05$). In OB rats, bone OPG/RANKL mRNA ratio was 0.39, a value below that in N (considered as unity), indicating a high resorptive activity in this model; treatment with either GLP-1 or Ex-4 increased this ratio to values close to normal (0.76 and 0.80, respectively).
Conclusion: These data suggest that both GLP-1 and Ex-4 not only could correct the high lipid levels present in obese state, but also to normalize its possible deleterious bone metabolism through their common anabolic action.
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The GLP-1 analogue liraglutide activates brainstem and hypothalamic neurons involved in appetite regulation

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Background and aims: The glucagon-like peptide-1 (GLP-1) analogue liraglutide is emerging as an important drug for the treatment of diabetes.

However, in both rodents and humans liraglutide also reduces appetite and lowers body weight.
Materials and methods: To identify the possible brain sites mediating liraglutide induced anorexia, we examined the expression of the immediate early gene c-Fos in male Sprague-Dawley rats. Based on the onset of liraglutide-induced anorexia, rats were terminated by perfusion fixation 4 and 8 h following s.c. or i.p. administration of liraglutide (0.1 mg/kg). Brain sections were immunoreacted for c-Fos and immunoreactive (ir) nuclei were assessed qualitatively throughout the brain. Six areas were selected for quantitative assessment.
Results: In the forebrain liraglutide increased c-Fos-ir nuclei in the central nucleus of amygdala (32±11; liraglutide s.c. 115±12; liraglutide i.p. 140±10, p<0.05 vehicle vs liraglutide s.c., i.p.) and in the hypothalamic paraventricular nucleus (PVN; vehicle 42±5; liraglutide s.c. 43±8; liraglutide i.p. 144±23, p<0.05 vehicle vs liraglutide i.p.). Conversely, liraglutide reduced c-Fos-ir nuclei in the arcuate nucleus (vehicle 45±10; liraglutide s.c. 16±4; liraglutide i.p. 21±6, p<0.05 vehicle vs liraglutide s.c., i.p.). In the hindbrain, liraglutide increased c-Fos in the area postrema (AP; vehicle 49±8; liraglutide s.c. 188±20; liraglutide i.p. 276±27, p<0.05 vehicle vs liraglutide s.c., i.p.), nucleus of the solitary tract (NTS; vehicle 27±5; liraglutide s.c. 150±17; liraglutide i.p. 161±21, p<0.05 vehicle vs liraglutide i.p.) and lateral parabrachial nucleus (lPBN; vehicle 13±4; liraglutide s.c. 87±15; liraglutide i.p. 117±13, p<0.05 vehicle vs liraglutide i.p., s.c.). Immunofluorescence was used to characterise liraglutide activated neurons. In the AP, liraglutide induced c-Fos in tyrosine-hydroxylase (TH)-ir neurons (vehicle 0±0; liraglutide s.c. 12.4±1.7; liraglutide i.p. 27.5±4%, p<0.05 vehicle vs liraglutide s.c., i.p.), whereas neither GLP-1-ir nor TH-ir neurons were activated in the NTS. In the PVN, liraglutide induced c-Fos in corticotrophin-releasing hormone-ir (CRH) neurons (vehicle 2±1; liraglutide s.c. 16±5; liraglutide i.p. 79±7%, p<0.05 vehicle vs liraglutide s.c., i.p.).
Conclusion: The data demonstrate that liraglutide activates the functional continuum involved in appetite regulation extending from the AP/NTS complex via the lPBN and amygdala to the PVN.
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Extended, 3-year, exenatide therapy shows sustainable effects on beta cell disposition index in metformin treated patients with type 2 diabetes
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Background and aims: We have previously showed that exenatide improved beta-cell function after 1-year treatment, relative to insulin glargine, with a similar glucose lowering effect. However, this effect was not sustained after a 4-week off-drug period. Here we report the effects on beta-cell function, presented relative to whole body insulin sensitivity as the disposition index, after the 2-year extension study.
Materials and methods: Sixty-nine metformin-treated patients with type 2 diabetes (mean±SD: age 59±8 y; HbA1c 7.5±0.8%; BMI 31±4 kg/m2; body weight 91.5±13.1 kg) were randomised to exenatide (n=36; 5µg BID for 4 weeks, 10µg BID with treat to HbA1c target ≤7%, maximum dose 20µg TID thereafter) or insulin glargine (n=33; treat to fasting plasma glucose (FPG) target <5.6 mmol/L). Forty-seven patients entered the 2-year open-label extension study of which 36 completed (exenatide n=16; insulin glargine n=20). Insulin sensitivity (M) and beta-cell function was measured by a combined euglycaemic-hyperinsulinaemic and arginine-stimulated hyperglycaemic clamp at baseline, and after a 4-week off-drug period, following the total 3-year treatment period. 1st-phase glucose stimulated C-peptide secretion was adjusted for the insulin-stimulated whole body glucose uptake (M) and presented as the disposition index (DI; AIRgluc*M).
Results: Both therapies reduced HbA1C similarly: by -0.7±0.3%, and -0.5±0.2% to 6.6±0.2% and 6.9±0.2% for exenatide and insulin glargine, respectively, after 3-years of treatment (P=0.186). Exenatide significantly reduced body weight compared to insulin glargine (between group difference: -7.9±1.8 kg; P<0.001), whereas treat to fasting plasma glucose insulin glargine more effectively lowered FPG (between group difference: -1.8±0.4 mmol/L; P<0.001). After the 4-week off-drug period, exenatide increased M by 39% (p=0.006) while insulin glargine had no effect on M (p=0.647). Following the 4-week off-drug period the DI was, compared to pre-treatment, higher with exenatide, and reduced with insulin glargine (+1.43±0.78 and -0.99±0.65, respectively; P=0.028). These findings are in contrast to the results following the 1-year treatment period, after which the DI did not show a sustained effect after the 4-week washout with either exenatide or insulin glargine. 2nd-phase glucose-stimulated, and combined glucose and arginine-stimulated C-peptide secretion did not show any significant between-group differences.
Conclusion: Exenatide and insulin glargine sustained HbA1C over 3-year treatment, while exenatide significantly reduced, and insulin glargine increased body weight. 1st-phase insulin secretion was sustained following a 4-week off-drug period, after 3-year treatment with Ex. This improvement cannot only be explained by glucose lowering.
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Molecular mechanism of DPP-IV inhibitor vildagliptin effect on pancreatic beta cell preservation in diabetic mice: evidence for anti-oxidative and ER stress mechanism
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Background and aims: DPP-IV inhibitors are known to produce several biological actions including glucose-dependent insulin secretion, increase pancreatic beta cell mass by stimulating cell proliferation and inhibit apoptosis. The aim of this study is to assess the molecular mechanism for preventive effect of vildagliptin on beta cell damage in diabetic animal model.
Materials and methods: Eight week-old male KK-*A*^{y}/TaJcl mice received vildagliptin (VILDA) (50mg/kg once daily orally) or vehicle (control) for 4 weeks (n=5). Body weight (BW), food intake, fasted blood glucose (FBG), fasted insulin (FIRI), TG and NEFA were measured at 8, 10 and 12 weeks of age. Intraperitoneal insulin tolerance test (ipITT:1 IU/kg BW), oral glucose tolerance test (OGTT:1 g/kg BW) and glucose stimulated insulin secretion (GSIS) from isolated pancreatic islet was performed at 12 weeks. The beta cell mass and cell proliferation were assessed by histological analysis including PCNA immunostaining of the islet tissue. Gene expressions specific for the core area of pancreatic islet were analyzed by Laser Capture Microdissection method and real time RT-PCR. Primer pairs encoding genes associated with pancreatic hormones, cell proliferation, apoptosis, cell cycle, and oxidative/endoplasmic reticulum (ER) stress were prepared, and real-time RT-PCR with Sybr Green was applied.
Results: BW, food intake, FBG, FIRI, HOMA-IR, TG, NEFA, and ipITT were not different between the control and VILDA-treated groups. On the other hand, VILDA ameliorated glucose tolerance analyzed by OGTT, and a significantly higher plasma insulin response to glucose challenge was observed in VILDA group compared with the control group. Furthermore, GSIS with 16.7mM glucose was more significantly facilitated in VILDA group than in the control (2.24±0.72 vs. 1.57±0.42 ng/ml/islet, $p < 0.05$). The pancreatic beta cell mass was greater in VILDA-treated mice than in the control mice (5.0±0.9 vs. 2.9±0.9mg, $p < 0.01$, n=5 for each). The mRNA levels of Nkx2.2, Pax6, ERK1 and CyclinD associated with cell differentiation/proliferation were significantly higher in VILDA-treated mice than in the control mice. CAD and caspase3 mRNA levels were significantly decreased by VILDA treatment. VILDA significantly down-regulated XBP-1 gene expression related with ER stress, and up-regulated GSHPx gene expression related with anti-oxidative stress. The PCNA-positive cell ratio in the islet was increased significantly by VILDA treatment. On the other hand, VILDA decreased the 4-HNE-positive cell ratio. Morphometric results for PCNA and 4HNE observed corresponded with the data obtained in gene expression analysis.
Conclusion: Vildagliptin preserves the pancreatic beta cell function and cell mass in diabetic KK-*A*^{y} mice. The present results suggest that the effect of vildagliptin is resulted not only from the direct action on the cell kinetics regulation but also from the suppression of oxidative and/or ER stress mechanism.

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Restoration of insulin release with lixisenatide in patients with type 2 diabetes

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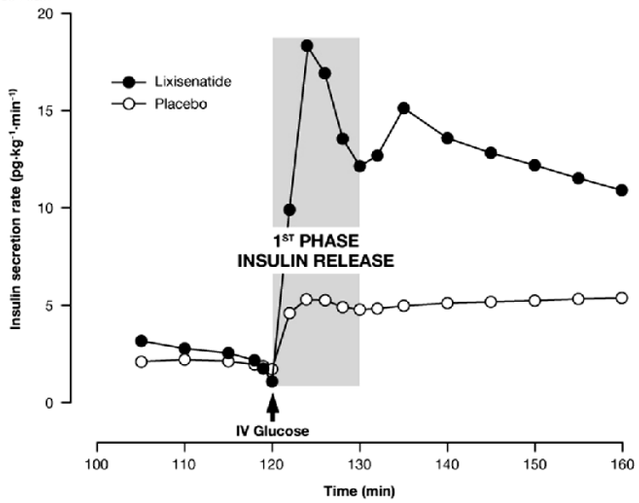
Background and aims: Glucagon-like peptide (GLP-1) receptor agonists improve post-meal blood glucose levels in subjects with type 2 diabetes by restoring insulin release. We assessed the pharmacokinetics and pharmacodynamics of lixisenatide, a new GLP-1 receptor agonist.
Materials and methods: A two-period, two-sequence, single-centre, single-dose, cross-over study was performed. Twenty subjects with type 2 diabetes (treated with metformin or diet and exercise alone) with mean HbA_{1c} of 6.8% (range = 6.2-8.0%) and mean BMI of 30 kg/m² (range = 26-35 kg/m²) received single s.c. doses of 20 µg lixisenatide once-daily (intended therapeutic dose) or placebo injection 2 h prior to an intravenous glucose challenge (IVG; 0.3 g/kg body weight over 30 s). Baseline blood glucose was lowered to 5.5 mmol/L by fasting or insulin infusion (4 subjects) 30 min before the IVG. First ($AUC_{0\text{-}10min}$) and second ($AUC_{10\text{-}120min}$) phase insulin secretion, plus C-peptide and glucagon release and glucose disposition rate (K*glu*) were assessed and lixisenatide exposure was measured over 12 h.
Results: The maximum lixisenatide plasma concentration (C_{max}) was 84 pg/mL (coefficient of variation [CV], 25%), with a mean time to maximum concentration of 2 h (range 1-4 h). Lixisenatide 20 µg s.c. enhanced first-phase insulin secretion to IVG by 2.8-fold relative to placebo (90% CI 2.5-3.1), and second-phase secretion by 1.6-fold (90% CI 1.4-1.7) (Figure); corresponding changes were seen in insulin (1st-phase: 6.6-fold; 90% CI 5.0-8.7 and 2nd-phase: 3.0-fold; 90% CI 2.7-3.3) and C-peptide concentrations and glucose disposition (accelerated by 1.8-fold; 90% CI 1.6-1.9). Lixisenatide had little effect on basal insulin and glucose levels and did not affect glucagon suppression. Mild-to-moderate gastrointestinal symptoms (e.g. nausea and vomiting) were reported by two subjects following lixisenatide administration and one following placebo.
Conclusion: Lixisenatide 20 µg s.c. restored insulin release and accelerated glucose disposition following an IVG in subjects with type 2 diabetes, confirming this action as the basis of its control of postprandial blood glucose levels.



Supported by: sanofi-aventis

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Liraglutide improves two indicators of beta cell function - HOMA-B and proinsulin:insulin ratio - in a meta-analysis of 6 clinical trials

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Background and aims: The once-daily human GLP-1 analogue liraglutide reduced HbA_{1c} by 1.0-1.5% during the phase 3 development programme. A meta-analysis of 6 large phase 3 trials was conducted to investigate if HbA_{1c} reductions were associated with improvement in HOMA-B, a surrogate marker of beta-cell function based on fasting insulin and glucose levels, and proinsulin-to-insulin ratio. Failing beta-cells secrete an abnormally high amount of proinsulin relative to insulin, reflecting incomplete or defective proinsulin processing.
Materials and methods: The effect of 26 weeks of treatment with liraglutide (1.2 mg/1.8 mg OD), rosiglitazone (4 mg OD), glimepiride (4 mg/8 mg OD), exenatide (10 µg BID) or placebo on HOMA-B and proinsulin:insulin ratio was examined using an ANCOVA model adjusted for treatment, trial, prior treatment and baseline HOMA-B and proinsulin:insulin ratio.
Results: A significant increase from baseline in HOMA-B was observed with liraglutide and with glimepiride (both p<0.0001). The increase in HOMA-B was significantly greater with liraglutide 1.8 mg vs. exenatide, rosiglitazone and placebo (Table). Significant decreases from baseline in proinsulin:insulin ratio were observed with liraglutide (p<0.0001) and exenatide (p<0.001). Decreases in proinsulin:insulin ratio were significantly greater with liraglutide vs. rosiglitazone, glimepiride and placebo (Table). Liraglutide and glimepiride increased HOMA-B to a similar extent. However, these two agents can be differentiated on the basis of their effects on proinsulin:insulin ratio. Liraglutide stimulates insulin secretion in a glucose dependent manner while glimepiride produces a continuous, glucose-independent signal, potentially resulting in constant stress on beta-cells.
Conclusion: Liraglutide improves HOMA-B and proinsulin:insulin ratio, two indicators of beta-cell function. Since beta-cell function is a primary determinant of type 2 diabetes progression, it is possible that liraglutide may alter the decline in beta-cell function seen in patients with this disease. However, long-term studies will be necessary to confirm this effect.

Mean change in HOMA-B and P/IR from baseline to week 26

Treatment	Δ HOMA-B (%)	Δ P/IR
Liraglutide 1.8 mg OD (n=1363)	35.1	-0.08
Liraglutide 1.2 mg OD (n=896)	31.7	-0.08
Rosiglitazone 4 mg OD (n=231)	9.5**	-0.01*,††
Glimepiride 2-4 mg OD (n=490)	31.8	-0.02*,††
Exenatide 10 µg BID (n=231)	5.7*	-0.10
Placebo (n=524)	7.4*,†	0.03*,†

*p<0.0001 and **p<0.05 vs. liraglutide 1.8 mg; †p<0.0001 and ††p<0.001 vs. liraglutide 1.2 mg

Supported by: Novo Nordisk

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Comparison between exenatide and glimepiride on metabolic control and on insulin resistance in type 2 diabetic patients with metformin therapy

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Background and aims: Our study aimed to compare the effect of Exenatide (Ex) vs Glimepiride (Gl) on glycemic control and on insulin resistance related-parameters in type 2 diabetic patients taking metformin.
Materials and methods: One hundred and thirty type 2 diabetic patients with uncontrolled type 2 diabetes [glycated hemoglobin (HbA_{1c} > 8 %)] were

randomised to Ex 5 μg b.i.d. or Gl 1.0 mg t.i.d. and titrated after 1 month to Ex 10 μg b.i.d. or Gl 2 mg t.i.d. They were resulted intolerant to metformin at maximum dosage (3000 mg/day) and were taking various different doses (1000-2000 mg/day). The treatment period had a 12 months duration. We evaluated body mass index (BMI), HbA_{1c}, fasting plasma glucose (FPG), post-prandial glucose (PPG), fasting plasma insulin (FPI), Homa index and collected plasma samples of adiponectin (ADN), and tumor necrosis factor-α (TNF-α) at baseline, and after 12 months.
Results: One hundred and eleven patients completed the study (57 in Ex and 54 in Gl group). BMI was significantly reduced by Ex, but not by Gl (from 28.4±1.3 to 26.6±0.9 Kg/m^2, p< 0.05, and from 28.5±1.4 to 28.2±1.3 Kg/m^2, ns vs baseline, p< 0.05 vs Ex, respectively). HbA_{1c} was decreased by 1.2±0.06 % (p< 0.01), and by 1.4±0.05 % (p< 0.01); FPG was reduced by 27±6 mg/dl (p< 0.01), and by 28±7 mg/dl (p< 0.01); PPG was decreased by 45±8 mg/dl (p< 0.01), and by 46±9 mg/dl (p< 0.01), in Ex and Gl group, respectively. FPI was decreased by 5.0±0.6 μU/ml (p< 0.05) in Ex group, and was increased by 1.2±0.09 μU/ml in Gl group (ns vs baseline, p< 0.05 vs Ex). Homa index was reduced by 2.7±0.8 (p< 0.05), and by 0.7±0.1 (ns vs baseline, p< 0.05 vs Ex), in Ex and Gl group, respectively. ADN was increased by 1.5±0.4 μg/ml (p< 0.05), and by 0.8±0.03 μg/ml (ns vs baseline, p< 0.05 vs Ex), in Ex and Gl group, respectively; TNF-α was reduced by 0.7±0.04 ng/ml (p< 0.05), and by 0.2±0.01 ng/ml (ns vs baseline, p< 0.05 vs Ex), in Ex and Gl group, respectively. There was a significant correlation between BMI value decrease and ADN increase (r= -0.57, p< 0.01), and TNF-α decrease (r= 0.61, p< 0.01).
Conclusion: Both Ex and Gl improved diabetes control when added to metformin, but only Ex improved insulin resistance related-parameters. The ADN increase, and TNF-α reduction seems to be related to weight loss.

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Treatment of type 1 diabetic patients with residual beta cell function with the once-daily glucagon-like peptide-1 analogue liraglutide
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Background and aims: Information about the effects of treatment with GLP-1 receptor analogues in people with type 1 diabetes is sparse. Therefore, we investigated the effect of the once-daily GLP-1 analogue liraglutide on daily insulin dose, 24-hour glucose profile, glycaemic control, weight change and side effects in type 1 diabetic patients with residual beta cell function.
Materials and methods: Eight type 1 diabetic patients with HbA1c 6.6 ± 0.3 %, male/female ratio 7/1, age 27 ± 1.6 yr, BMI 24.3 ± 1.0 kg x m^{-2}, diabetes duration 3.7 ± 0.8 yr, and with plasma C-peptide concentration after 1 mg glucagon of 472 ± 107 pmol/l (range: 79-940) performed at a blood glucose of 15 mM, were treated for four weeks with liraglutide (0.6 mg for one week hereafter 1.2 mg daily if tolerated). All patients were treated with basal/bolus insulin therapy. Before initiation of liraglutide treatment, glycaemic control was optimized, and throughout the study patients were encouraged to maintain the best possible glycaemic control taking the risk of hypoglycaemia into consideration. Glycaemic control was evaluated during three days before start of liraglutide and the last three days of treatment through HbA1c, fructosamin and with patient blinded Guardian monitoring with logbook of meals and physical activity.
Results: Daily dose of insulin was significantly reduced from 0.53 ± 0.1 to 0.33 ± 0.1 U/kg/day, p< 0.001 during treatment with liraglutide despite no change in mean blood glucose, which was 6.1 ± 0.3 and 6.3 ± 0.4 mmol/l before and during the last three days of treatment, p=NS. Two patients completely discontinued their insulin treatment without loss of glycaemic control. The percentage of plasma glucose measurements below 3.9 mmol/l significantly decreased from 12.0 ± 4 to 5.1 ± 2, p=0.03. HbA1c decreased during treatment from 6.6 ± 0.3 to 6.2 ± 0.2, p=0.05 whereas no change was observed for fructosamin. All patients lost weight with a mean of -2.6 kg (range -1.5 to -3.6). Six patients had minor nausea, which was mostly transient, two patients experienced vomiting and four patients complained of abdominal pain and distension. Two patients tolerated only 0.9 mg liraglutide daily because of side effects.
Conclusion: Treatment with liraglutide in people with type 1 diabetes with residual beta cell function, may reduce the daily dose of insulin with improved or unaltered glycaemic control evaluated from fructosamin, HbA1c and mean glucose level evaluated during 3 days of Guardian monitoring. In type 1 diabetic patients with considerable residual insulin secretion, insulin treatment may be discontinued without impairment of glycaemic control. All patients lost weight during liraglutide treatment. Gastrointestinal side effects occurred frequently, but were mostly transient.

PS 77 GLP-1 analogues: safety and monitoring

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Increased collagen production of human hepatic stellate cells (Ito Cells) induced by Exendin-4 (Exenatide) treatment
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Background and aims: There are precautions about the potential for acute pancreatitis in patients taking Exenatide and these concerns were confirmed in experimental animal studies. Provided that a proportion of active glucagon-like peptide-1 (GLP-1) hormone also reaches the hepatic portal tract via the superior mesenteric vein after release form the small intestine we hypothesized that a hepatic effect should also exist even under physiologic conditions. We studied the potential effect of Exenatide on extracellular matrix (ECM) production of hepatic stellate cells (HSCs) that are thought to primarily produce the ECM proteins in the liver. These cells with fibrogenic potential were treated and co-treated with Exenatide and TGF- β1.
Materials and methods: Immortalized human HSC LX-2 cells were cultured at 37 °C atmosphere containing 5% CO2 with DMEM containing 10% FBS. Passages were made after 3-4 days using trypsin-EDTA. Cells were then cultured for 48 hours in FBS-free media that in the treated groups either contained Exenatide (cc=200 pg/mL) or TGF-β1 (cc=2ng/mL) or both (co- treatment). After mRNA isolation and RT-PCR Taq-Man real-time PCR assays were run in ABI 7000 System and human 18S rRNA was used for normalization. To evaluate the ECM production of HSCs ELISA assay was performed from the culture media using rabbit polyclonal primary antibodies against collagen-I and collagen-III. ECM protein synthesis was also assessed using Western Blots. Immun-cytochemistry (αSmooth Muscle Actin) was used to prove the activation of HSCs. All treatments were repeated 3 times and all runs were run in duplicates. Two-tailed T-test was used for statistical analysis.
Results: ECM protein production results measured by ELISA in the culture media are indicated in the following table (*= significantly increased compared to untreated controls, * * = significantly increased compared to TGF- β1 treatment). The collagen-III production of HSCs after Exendin-4 treatment was also assessed by Western blots that confirmed the ELISA results (collagen-I production was more preponderant after TGF-β1 treatment on Western Blot compared to ELISA) . The alterations of mRNA expression levels were reflecting the changes observed at protein level (upregulation) although to a less remarkable extent than the degree of change in collagen production.
Conclusion: These data confirm the existence of an incretin mediated entero-hepatic axis. Specifically we intended to uncover the distinct nature of the GLP-1 mimetic Exendin-4 and its effect on Hepatic Stellate Cells with fibrogenic potential. The surprising induction of human HSC collagen-III production by Exendin-4 treatment established a novel path of regulating the ECM production in the liver and urges further clinical trials not only to clarify the pancreatic effects but also with hepatic fibrosis and chronic liver disease endpoints.

Treatment	**Collagen 1** (fold change)	SD
Exendin-4	**1.7***	0.13
Exendin-4+TGF-B1	1.07	0.24
TGF-B1	**1.73***	0.18
Untreated Control	1.0	0.28

	Collagen 3 (fold change)	SD
Exendin-4	**16.31*,****	0.75
Exendin-4+TGF-B1	**16.18*,****	0.81
TGF-B1	**3.19***	0.31
Untreated Control	1.00	0.06

Supported by: Hungarian Diabetes Association

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Liraglutide protects against traumatic brain injury in a mouse model

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Background and aims: Glucagon-like peptide-1 (GLP-1) analogues are emerging as an important drug class for the treatment of diabetes because they not only lower blood glucose but also body weight. Recently, several articles have pointed to a protective effect of GLP-1 or analogues on the brain and potential efficacy in animal models of stroke, Alzheimer's, Huntington's and Parkinson's disease. However, in some cases the compounds were dosed by the intracerebroventricular route. We here report the effect of liraglutide treatment during traumatic brain injury (TBI) in a mouse model. Liraglutide is a once-daily human GLP-1 analogue, the first to give 24 hours' coverage in patients. Since the effects in the brain are mediated at least in part by a protective mechanism, 24-hour coverage could be important.
Materials and methods: Liraglutide was injected s.c. (0.4 mg/kg) twice daily before and after mice received a cryo-induced cortical lesion until 7 days post-lesion. Control mice received a match volume of s.c. saline. Mice were processed for immunohistochemistry.
Results: When compared to the saline-treated controls, liraglutide-treated mice had less inflammation as reflected by a decrease in lectin + microglial activation. In liraglutide-treated mice 8-oxoguanine DNA adduct formation was reduced and largely specific to endothelial cells, whereas in saline-treated mice adducts were widespread through the lesion and present primarily in neurons, microglia and endothelial cells. Liraglutide improved tissue-healing response in TBI mice through concerted, lesion-directed astrogliosis, increased migration into the lesion and meninges formation, and improved blood-brain barrier reconstitution through reduced albumin leakage and increased perivascular astroglia.
Conclusion: Our data demonstrate that the GLP-1 analogue liraglutide promotes an anti-inflammatory, antioxidant state in a murine traumatic brain injury model and improves tissue healing and blood-brain barrier reconstitution. These findings warrant further investigation into the mechanisms of action and future studies will explore the potential of liraglutide in protecting against neuropathology.
Supported by: Novo Nordisk

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The GLP-1 analogue liraglutide does not induce pancreatitis in mice, rats or monkeys

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Background and aims: GLP-1 receptor agonists may be associated with an increased risk of pancreatitis in patients with type 2 diabetes. However the low overall incidence of pancreatitis and a 3-fold increase in pancreatitis rates in diabetes patients makes it difficult to assess if the association is true. Phase 3 studies with liraglutide in 4400 type 2 diabetes patients did not demonstrate a clear association between diabetes treatment and pancreatitis. A mechanism linking GLP-1 receptor activation to pancreatic inflammation has not been forthcoming.
Materials and methods: We report pancreas safety data in mice, rats and monkeys during the non-clinical development programme for liraglutide. Pancreases from mice, rats and cynomolgus monkeys were examined macro- and microscopically using state-of-the-art diagnostic criteria. Mice and rats were dosed for 2 years, monkeys 87 weeks (n=66-79/sex/group for mice, n=50 for rats, n=5 for monkeys; doses up to 3, 0.75, and 5 mg/kg/day, respectively in mice, rats and monkeys). Proliferation was measured using PCNA staining in rats after 26 weeks. The evaluation in monkeys included detection of signs of pancreatic intraepithelial neoplasia in the ductal epithelium.
Results: There were no macroscopic observations of pancreatitis in mice, rats or monkeys. After 2 years treatment, 8 out of 359 male (3 in the control group and 2, 1, 1, 1 in the different liraglutide groups) and 12 out of 354 female mice (0 in the control group and 3, 3, 3, 3 in the liraglutide groups) were diagnosed microscopically with pancreatitis based on histological criteria of inflammatory infiltrates with or without fibrosis and/or loss of exocrine tissue. Pancreatitis was not the cause of death in any of these animals and pancreatitis is seen spontaneously in mice. There were no cases of pancreatitis in 400 male and female rats, after 2 years dosing. Cell proliferation in the exocrine pancreas was not increased in rats dosed with liraglutide for 26 weeks. Neither pancreatitis nor pre-neoplastic proliferative lesions were found in monkeys dosed for 87 weeks, resulting in plasma liraglutide exposure 60-fold higher than that observed in humans at the maximal clinical dose.
Conclusion: In conclusion, liraglutide does not induce pancreatitis in rats or mice dosed for 2 years or in non-human primates dosed for 87 weeks.
Supported by: Novo Nordisk

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The incidence of antibody formation and the levels of antibodies are lower with liraglutide than exenatide in a head-to-head comparison

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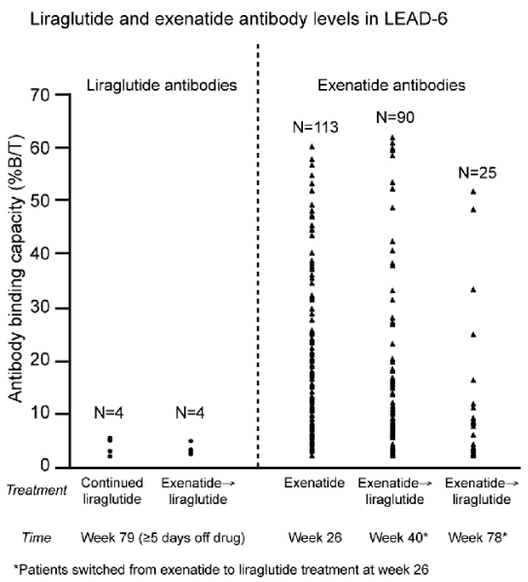
Background and aims: Antibody formation to therapeutic proteins can potentially affect pharmacokinetics, trigger adverse events, and/or diminish clinical response. Liraglutide (LIRA), a human GLP-1 analog, and exenatide (EXE), synthetic exendin-4 (a peptide originally identified in Gila monster saliva), are GLP-1 receptor agonists indicated for treatment of T2D with 97% and 53% homology to human GLP-1, respectively. Our objective was to detect and characterize antibodies to LIRA and EXE.
Materials and methods: LIRA and EXE antibodies were measured at wks 0, 12, 26, 40, 41, 78 and 79 in the LEAD-6 trial, which had a 26-wk head-to-head comparison of LIRA (1.8mg, once daily) and EXE (10µg, twice daily before meals), both + met and/or SU, followed by a 52-wk extension when EXE-treated patients switched to LIRA. Fasting samples collected prior to administration of trial products were screened for LIRA or EXE antibodies using a validated radioimmunoprecipitation assay. Plasma LIRA (30nM) reduced the sensitivity of the assay to detect low LIRA antibody levels from 25-50ng/mL to ~1500ng/mL. Thus, to avoid assay interference with plasma LIRA, LIRA antibody values presented were from end of trial after a ≥5-d (~7-9*$t_{1/2}$) wash-out. Because of its ~100-fold lower plasma concentration and short half-life (2.4h), plasma EXE levels were below the level of interference with the assay at the time samples were collected; therefore, EXE antibodies could be accurately measured during treatment.
Results: Of 467 patients, 389 (83%) entered the extension and 299 (64%) completed 78 wks. After 78 wks on LIRA, 4/154 (2.6%) patients off drug ≥5 d had low-titer LIRA antibodies (Fig, range: 1.9-5.3% Bound/Total [B/T]). HbA_{1c} changes in these 4 patients were 0.4, 0.8, -1.4 and -1.9% at 78 wks. After 26 wks on EXE, 113/185 (61%) extension patients had EXE antibodies (Fig, range: 2.4-60.2%B/T). EXE patients with high antibody titers (>20%B/T) had smaller HbA_{1c} reductions (-0.5%, N=47) than those with low titers (-1.0%, N=132). Patients with the highest EXE antibody titers (>50%B/T, N=6) had a -0.1% HbA_{1c} reduction. After switching from EXE to LIRA at wk 26, 90/181 (50%) and 25/143 (18%) patients had EXE antibodies at wks 40 and 78, respectively. Persistent EXE antibodies did not appear to affect glycemic efficacy with LIRA. Only 4/134 (3.0%) patients who switched from EXE to LIRA for 1 yr had LIRA antibodies.
Conclusion: LIRA resulted in a much lower frequency and magnitude of antibody formation than EXE. High-titer EXE antibodies affected glycemic response to EXE, but after switching to LIRA, persistent EXE antibodies did not compromise glycemic response to LIRA.



Supported by: Novo Nordisk

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Antibodies to exenatide did not cross-react with human GLP-1 or glucagon or alter the efficacy or safety of exenatide

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Background and aims: Consistent with the immunogenic properties of protein and peptide pharmaceuticals, patients may develop antibodies to exenatide with exenatide treatment. This analysis characterised the time course and cross-reactivity of antibodies produced against exenatide and effects on efficacy and safety.
Materials and methods: Antibody titers, and glycaemic and safety parameters were frequently sampled in 12 long-term controlled (2225 ITT patients, 12-52 wk duration) and 5 long-term uncontrolled (1538 ITT patients, up to 3 y) exenatide BID (Ex BID) trials and 4 long-term controlled (653 ITT patients, 26-30 wk) exenatide once weekly (Ex QW) trials.
Results: For Ex BID, mean antibody titers peaked between wks 6 and 16, and were reduced by 39.4% and 65.2% at wks 30 and 52, respectively. At wk 30, 37% of patients were positive for antibodies to exenatide. Of those, 32% exhibited low antibody titers (≤125) and a minority (5%) had higher antibody titers (≥625). At wk 52, 25% of patients were antibody positive (3% higher titer), and at 3 y, 17% were positive (1% higher titer). Lower titer antibodies had no effect on efficacy as evidence by comparable reductions in HbA_{1c} at endpoint in controlled trials (-1.0% for both antibody-negative and low-titer subjects). In the small number of patients with higher titers (5%), the impact on efficacy was variable, with the majority experiencing a glycaemic response consistent with antibody-negative patients. Similar trends were observed at 1 and 3 y. Other than injection-site reactions, no increased incidence of adverse events was observed with antibodies to exenatide. Cross-reactivity was examined in vitro for a subset of patients (106 antibody-positive patients), and treatment-emergent antibodies to exenatide did not cross-react with human GLP-1 or glucagon. Consistent with Ex BID, patients treated with Ex QW experienced titers that peaked between wks 6 and 16 and subsequently declined, with 56.8% of patients antibody positive (11.8% higher titer) at wk 26-30 and similar consequences on efficacy and safety.
Conclusion: Although patients may develop antibodies to exenatide, the titers peak early in treatment and decline thereafter, and are not predictive of safety and efficacy. Importantly, as antibodies to exenatide do not cross-react with the glucoregulatory hormones GLP-1 or glucagon and diminish over time, long-term clinical consequences are unlikely.
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Relevance of sample collection method and specificity for the quantification of GLP-1-1 in two new ELISA assays for active and total GLP-1

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Background and aims: The processing of proglucagon in the pancreas and small intestine give rise to a number of different peptides, including GLP-1. Intestinal derived GLP-1 (7-37) and (7-36)amide (often called active GLP-1) are rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-IV) to GLP-1 (9-37) and (9-36)amide, resulting in very low amount of active GLP-1 in the circulation. The immunological determination of GLP-1 in a plasma sample is complicated by the potential cross reaction of the various proglucagon-derived peptides with antibodies raised against GLP-1 and the low amounts and rapid degradation of active GLP-1. In this study we investigated different sample collection methods including different inhibitors of the DPP-4 enzyme in two novel ELISA assays for GLP-1. The specificity for different GLP-1 isoforms were also determined.
Materials and methods: The crossreaction of GLP-1 (7-36)amide, (1-36)amide, (1-37), (7-37) and (9-36)amide were analyzed in two newly developed GLP-1 ELISAs for active and total GLP-1. Blood samples were collected from 9 healthy donors in 4 different EDTA tubes at the same occasion. Tube types 1 and 2 were precoated with lyophilized inhibitors (1 = DPP-IV inhibitors and 2 = A cocktail of protease, esterase and DPP-IV inhibitors). Tube 3 contained no DPP-IV inhibitor. A liquid DPP-IV inhibitor was added immediately after blood collection to tube 4. Plasma was prepared and the 4 sample types were then analyzed in the active and total GLP-1 ELISA assays.
Results: The GLP-1 active ELISA is specific for GLP-1 (7-36)amide (100% crossreaction) and do not detect GLP-1 (7-37), (1-37) or (9-36) (<0.4% cross-reaction). The crossreaction for GLP-1 (1-36)amide was 3.5%. The GLP-1 total ELISA is specific for GLP-1 (1-36)amide, (7-36)amide and (9-36)amide but do not detect non-amidated forms of GLP-1. When the 4 different sample types were analyzed in the GLP-1 active ELISA, tube 3 (EDTA only) showed a significantly lower content of GLP-1. There were no differences in GLP-1 content between the different inhibitors used in tubes 1, 2 and 4. (mean±SEM 2.1±0.3 pM for tube 3 vs. 4.5±0.7 pmol for tube 1, 4.6±0.7 pM for tube 2 and 4.5±0.8 pM for tube 4, $p < 0.002$, ANOVA). When samples were analyzed for total GLP-1, tube 2 contained somewhat higher amounts of total GLP-1 compared to tubes 1, 3 and 4 (mean±SEM 13.7±1.7 pM for tube 2 vs. 12.6±1.6 pmol for tube 1, 12.8±1.6 pM for tube 3 and 12.2±1.5 pM for tube 4, $p < 0.001$, ANOVA).
Conclusion: The ELISA for active GLP-1 is specific for GLP-1 (7-36)amide. The total GLP-1 ELISA was shown to be specific for all amidated isoforms of GLP-1. For detection of active GLP-1, it is important to include a DPP-IV inhibitor to prevent degradation. Both lyophilized and liquid DPP-IV inhibitors gave comparable results in preserving active GLP-1 in a plasma sample. DPP-IV inhibition does not seem to be essential for preservation of total amidated forms of GLP-1, but addition of protease and esterase inhibitors seem to induce some protection from degradation of total GLP-1 in a plasma sample.
Supported by: Mercodia AB

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Liraglutide: short-lived effect on gastric emptying - long-lasting effects on body-weight

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Background and aims: Previous studies with the novel once-daily GLP-1 analogue liraglutide and the GLP-1 mimetic exenatide have revealed profound insulinotrophic and anti-diabetic effects, but also long-term and lasting reductions in body-weight. Considering the marked inhibitory effects of GLP-1 on gastric emptying (GE), it is tempting to speculate that inhibition of GE could play a major role in the body-weight-lowering effects of liraglutide and exenatide. However, data for liraglutide indicate that the marked inhibition of GE diminishes over time, and thus cannot be the sole mechanism for body weight lowering.
Materials and methods: The present study was designed to test the effect of acute and chronic exposure of liraglutide and exenatide on GE, food intake and body weight. Based on a series of dose-finding studies we identified doses of exenatide (0.01 mg/kg) and liraglutide (0.2 mg/kg) with similar anorectic effects. GE was assessed using a standard acetaminophen release assay. Acetaminophen (40 mg/kg) was administered by gavage 30 min after an intravenous dose of exenatide and liraglutide. Rats were subsequently subcutaneously dosed bi-daily for 14 days after which GE was assessed again 30 min following the final sc injection of compounds.
Results: While both compounds exerted robust acute reductions on GE (area under the curve (µg/ml x min); vehicle 8520±290, liraglutide 1088±214, exenatide 1488±315; $p<0.0001$ vehicle vs liraglutide, exenatide) the effects on GE almost disappeared following 14 days dosing with liraglutide (vehicle 9362±469, liraglutide 8135±380; $p=0.022$). In contrast, exenatide treated rats still displayed a profound reduction in GE at the 14-day time-point (exenatide 591±137 µg/ml x min; $p<0.001$). Both compounds exerted similar chronic effects on body-weight (vehicle 350±4 g, liraglutide 318±5 g, exenatide 303±8; $p<0.0001$ vehicle vs liraglutide, exenatide).
Conclusion: The data suggest that the 'gastric inhibitory' GLP-1 receptors in rats are subject to desensitisation only during full 24 h exposure as obtained by liraglutide, whereas the GLP-1 receptors mediating the effects on body-weight are not. These data indicate that regulation of appetite signals in the brain, and not gastric emptying, is the main mechanism for liraglutide induced weight loss.
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PS 78 Incretins and insulin studies

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Safety and efficacy of using exenatide in combination with insulin in the Association of British Clinical Diabetologists (ABCD) nationwide exenatide audit

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Background and aims: To learn from experience of exenatide in real clinical use in the UK, ABCD began a nationwide audit in December 2008. Though exenatide is not licensed for use with insulin, many contributors used the combination. We aimed to assess the extent, safety and efficacy of this off license usage.
Materials and methods: ABCD website hosted, password protected, on-line questionnaire for collection of anonymised patient data. Paired T tests compared baseline and latest weight and HbA1c. Hypoglycaemia reports were quantified.
Results: 315 contributors from 126 centres submitted data on 6717 patients - mean baseline age 54.9, HbA1c 9.47%, weight 113.83 kg, BMI 38.9 kg/m2, 55.5% male. Of these 4691 had dated baseline and latest HbA1c; 4506 dated baseline and latest weight. Latest HbA1c and weight were at a median (range) of 26.3 (6.6-164.1) and 26.1 (6.6-159.0) weeks respectively after exenatide start. Insulin treatment status was assessable in 6158/6717 (91.7%) patients. These were divided into 5 groups (see table). 2061/6158 (33.5%) were on insulin at exenatide start (groups 2-4). 1584/6158 (25.7%) continued on insulin (group 4). For those with dated baseline and latest data, the response of HbA1c and weight in the different insulin treatment groups are shown in the table. It can be seen that the addition of exenatide to insulin (group 4) was associated with significant falls of HbA1c and weight by 0.43% and 5.8 kg respectively. Insulin was stopped at the time of exenatide start in 477/2061 (23.1%) of those on insulin. Of these 325/477 (68.1%) substitution of exenatide for insulin proved highly effective with HbA1c falling by 0.69% and weight by 9 kg. However in 152/477 (31.9%) discontinuing insulin at the time of exenatide start resulted in significant deterioration in glycaemic control and restart of insulin. In total 2257/6165 (36.7%) experienced exenatide with insulin at some stage (groups 3-5). Of these 133/2257 (5.9%) experienced hypoglycaemia prior to insulin exenatide combination and 193/2257 (8.6%) after (p=0.001). However severe hypoglycaemia was reported in only 1/2257 (0.04%). 201/1584 (12.7%) of the patients who continued insulin at exenatide start (group 4) came off insulin during exenatide treatment. In this group both HbA1c and weight fell by considerable amounts: mean HbA1c by 0.81% from 9.4 to 8.59% (p<0.001) and weight by 10 kg from 116 to 106 kg (p<0.001).
Conclusion:
1. The combination of insulin with exenatide was used in 36.7% (2257/6158) patients in the ABCD nationwide exenatide audit.
2. Exenatide with insulin in real clinical use in the UK has been both safe and effective with significant reductions in both weight and HbA1c and only one reported case of severe hypoglycaemia.
3. Exenatide allowed some patients to be weaned off insulin and this group may experience a considerable improvement in glycaemic control and a large reduction in weight.

	HbA1c (%)			Weight (Kg)		
Group	Baseline HbA1c	Latest HbA1c	Fall in HbA1c	Baseline weight	Latest weight	Fall in weight
Group 1: Not on insulin (n=3576)	9.42	8.45***	0.98	114.4	109.0***	5.4
Group 2: Insulin stopped at start and stayed stopped (n=325)	9.74	9.05***	0.69	111.5	102.5***	9
Group 3: Insulin stopped at start but restarted (n=152)	9.73	9.92°	-0.18	108.5	101.3***	7.2
Group 4: Insulin continued at start (n=1584)	9.54	9.11***	0.43	112.6	106.8***	5.8
Group 5. Not on insulin at start but added later (n=521)	9.50	9.32*	0.18	114.5	107.8***	6.7

***p<0.001 vs baseline. *P<0.05 vs baseline. °ns vs baseline

Supported by: Eli Lilly Ltd

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Exenatide vs. insulin aspart in patients with type 2 diabetes: results of a randomised, open-label study

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Background and aims: Cardiovascular trials suggest that hypoglycemia (hypo) may be underestimated as risk factor for type 2 diabetes (T2DM). This 26wk, randomized, open label study was designed to test if exenatide (EX) is noninferior to insulin aspart (IA) for glycemic control, and associated with a lower hypo risk in patients (pts) pretreated with metformin (MET; primary objective, hierarchical test). Pts pretreated with MET+sulfonylurea (SU) were enrolled into an additional exploratory arm.
Materials and methods: Adults with T2DM (HbA_{1c} 6.5-10.0%, BMI 25-40 kg/m^2) pretreated with MET only (confirmatory) or MET+SU (exploratory) were enrolled at 3:1 ratio. Within each arm, pts were randomized 1:1 to EX (4wks 5µg, then 10µg BID) or premixed IA 70/30 twice daily (ADA targets). For noninferiority of EX, the upper 95% CI limit of the group difference in HbA_{1c} change (EX minus IA; MMRM, MET only) needed to be <0.4%. If noninferiority was met, hypo rates (pts with ≥1 documented [BG ≤3.9 mmol/l] or severe hypo up to wk26) were compared by Kaplan-Meier analysis (overlap of 95% CIs; all pts treated). Corresponding exploratory analyses were performed for the MET+SU arm.
Results: Of 494 pts randomized (363 MET only; 131 MET+SU), 480 started study drug (MET only: EX 181/IA 173; MET+SU: EX 66/IA 60). 341 pts completed the study (MET-only: EX 135/IA 137; MET+SU: EX 31/IA 38). Compared to pts pretreated with MET only, MET+SU pts were older (mean age [95% CI]: 60 [58.2; 61.8] vs 57 [56.0; 58.1] y, and had a longer diabetes duration (8 [7.3; 9.4] vs 5 [4.6; 5.5] y). Baseline BMI (32.0 [31.3; 32.8] vs 33.2 [32.7; 33.6] kg/m^2) and HbA_{1c} (7.98 [7.85; 8.12] vs 7.88 [7.79; 7.97] %) were similar. MET+SU pts required higher insulin doses (mean final dose [95% CI]: 0.43 [0.36; 0.50] vs 0.29 [0.27; 0.32] IU/kg/d). Within each pretreatment arm, characteristics of EX and IA groups were similar. For patients pretreated with MET only, glycemic control with EX was non-inferior to IA (LS mean difference at Wk 26: 0.14; 95% CI [-0.003; +0.291]); EX was associated with lower hypo rates (Table; p<0.05). For patients pretreated with MET+SU, glycemic control with EX was inferior (+0.80 [+0.41; +1.19]). The difference in hypo rates (Table) was not significant. Body weight decreased with EX and increased with IA irrespective of pretreatment (p<0.001).
Conclusion: In pts pretreated with MET only, EX was non-inferior to IA for glycemic control and superior for control of hypoglycemia and body weight (confirmatory arm). In the small exploratory arm pretreated with MET+SU, EX was inferior for glycemic control, this might be due to more advanced disease and therefore smaller beta cell reserve.

Table: Summary of results

		Pretreated MET only (confirmatory)		Pretreated MET+SU (exploratory)	
		EX (N=181)	IA (N=173)	EX (N=66)	IA (N=60)
HbA_{1c} (%)	LSmean change, wk26	-1.0	-1.1	-0.2	-0.9
	95% CI	-1.10; -0.89	-1.24; -1.04	-0.42; +0.13	-1.21; -0.67
Hypo rate(%)	Rate to wk26	8.0	20.5	12.4	27.0
	95% CI	4.7;13.4	15.0;27.7	6.4;23.3	16.8;41.8
Body weight (kg)	LSmean change, wk26	-4.1	+1.0	-4.9	+0.5
	95% CI	-4.5;-3.7	+0.6;+1.4	-5.6;-4.2	-0.2;+1.2

Supported by: Eli Lilly and Company and Amylin Pharmaceuticals, Inc

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Impact of exenatide once weekly and insulin glargine on glucose control and cardiovascular risk factors in subjects with type 2 diabetes

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Background and aims: Many patients failing to achieve blood glucose control on oral agents have elevated cardiovascular (CV) risk factors including hypertension and hyperlipidaemia. We examined glycaemic control (primary endpoint, change in HbA1c) and select CV risk factors (secondary endpoints) in subjects who were randomised to receive a glucagon-like peptide-1 receptor agonist (exenatide once weekly [EQW]) or a common starter insulin (insulin glargine [IG]) for 26 weeks.
Materials and methods: A 26-week, open-label, multicountry, randomised, superiority study compared EQW to IG titrated to target in 456 subjects with type 2 diabetes (mean HbA1c 8.3 [SD 1.1] %, fasting glucose 9.8 [2.6] mmol/L, weight 90.9 [17.5] kg, diabetes duration 7.9 [6.0] years, endpoint insulin dose 31 IU/day). Randomisation was stratified by country and oral blood glucose-lowering therapy (70% metformin alone; 30% metformin plus sulphonylurea). Changes in concomitant lipid-lowering and antihypertensive medications were allowed if deemed necessary by the investigator. Post hoc assessments were performed: 1) Change in CV risk factors in subjects with abnormal baseline (defined in Table 1) and; 2) Correlations between CV risk factors and body weight.
Results: EQW and IG both reduced HbA1c significantly from baseline (-1.5 [SE 0.05] % vs. -1.3 [0.06] % respectively, treatment difference -0.16 [0.07] %, p=0.017). The majority of subjects had elevated lipids and/or blood pressure at baseline (Table 1). EQW subjects experienced small but statistically significant improvements in total cholesterol and high-sensitivity C-reactive protein (hsCRP), whereas IG subjects experienced improvements in triglycerides and alanine aminotransaminase (ALT). In both treatment groups, greater improvements in blood pressure, fasting serum lipids, ALT, and hsCRP were observed in subjects with abnormal baseline values. Subjects treated with EQW lost body weight while IG subjects gained weight (-2.6 [SE 0.2] kg vs. 1.4 [0.2] kg, treatment difference -4.0 [0.3] kg, p<0.001). Body weight change was weakly correlated with change in ALT (EQW: adjusted r=0.03, p=0.008) and SBP (IG: adjusted r=0.02, p=0.03).
Conclusion: In this 26-week study, superior improvements in HbA1c and body weight were observed with EQW treatment relative to IG. Treatment with either EQW or IG for 26 weeks resulted in small but significant changes in different surrogate markers of cardiovascular risk. Subjects with abnormal baseline CV risk factors exhibited the greatest improvements in those parameters.

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Taspoglutide added to metformin provides comparable glycaemic control as insulin glargine with superior weight loss and less hypoglycaemia in type 2 diabetes: The T-emerge 5 Trial

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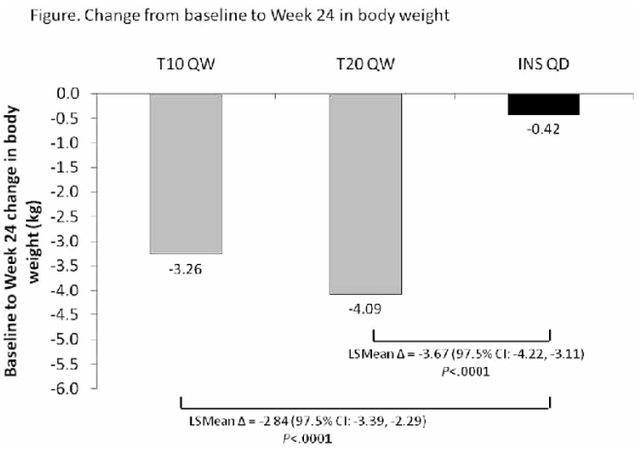
Background and aims: Taspoglutide is a once-weekly (QW) human GLP-1 analog in Phase 3 development for the management of type 2 diabetes (T2D). This trial evaluated a forward strategy replacing sulfonylureas (SU) by taspoglutide or insulin glargine in combination with previous metformin (MET) therapy.
Materials and methods: T-emerge 5 compared QW taspoglutide to once-daily (QD) insulin glargine (INS) in adult insulin-naïve patients with T2D inadequately controlled with MET+SU. After discontinuation of SU for 5±1 days, 1049 patients were randomized 1:1:1 to QW taspoglutide 10 mg (Taspo10), QW taspoglutide 20 mg (Taspo20; up-titrated after 4 weeks of 10 mg), or QD INS for 24 weeks. INS was adjusted using a forced titration algorithm to achieve optimal target fasting plasma glucose of 6.1 mmol/L (110 mg/dL). The primary endpoint was the change from baseline in HbA_{1c} at week 24. The analysis was conducted using the intent-to-treat population to test the non-inferiority of Taspo10 and Taspo20 with INS (using 2-sided 95% CI for HbA_{1c} difference and non-inferiority margin of 0.4%).
Results: Baseline characteristics were comparable across groups (mean values: age 58 yrs, BMI 32 kg/m^2, BW 91 kg, and HbA_{1c} 8.3%). LS mean (SE) change from baseline in HbA_{1c} was -0.8% (0.05), -1.0% (0.05) and -0.8% (0.05) for Taspo10, Taspo20 and INS groups, respectively, with both Taspo10 and Taspo20 being non-inferior to INS. Proportion of patients achieving HbA_{1c} ≤7.0% was greater for both Taspo arms compared with INS (Taspo10, 39%; Taspo20, 47%; INS, 32%). Taspo10 and Taspo20 produced significantly greater weight loss vs. INS (Figure). In addition, the proportion of patients who experienced hypoglycaemia (confirmed/unconfirmed) was significantly lower with Taspo10 (5%) and Taspo20 (6%) vs. INS (17%) (P<0.0001 for both). Serious AE incidence was similar among the groups. Withdrawals due to adverse events (AEs) were higher with Taspo vs. INS, primarily due to gastrointestinal (GI) AEs (Taspo10, 6%; Taspo20, 10%; INS, 0%).
Conclusion: Compared to daily INS, addition of once-weekly Taspo to previous MET in patients failing on MET+SU improved similarly glycaemic control with additional weight loss and less hypoglycaemia, but more GI AEs. (NCT00755287)

Table 1.

	SBP mmHg	DBP mmHg	LDL-C mmol/L	HDL-C mmol/L	TG mmol/L	TC mmol/L	ALT IU/L	hsCRP mg/L
Abnormal threshold	≥130	≥80	>2.6	<1.3/1.0†	>1.7	>5.2	>19/30†	>3
EQW: Mean BL (overall)	135 (1)	81 (1)	2.7 (0.1)	1.2 (0.0)	2.1 (0.1)	4.8 (0.1)	32 (1)	5 (1)
EQW: Week 26 Δ (overall)	-3 (1)*	-1 (1)	-0.1 (0.1)	0.0 (0.0)	-0.1 (0.1)	-0.1 (0.1)*	-2 (1)	-2 (1)*
EQW: Week 26 Δ (BL abnormal)	-6 (1)*	-5 (1)*	-0.2 (0.1)*	0.06 (0.01)*	-0.5 (0.1)*	-0.4 (0.1)*	-7 (2)*	-3 (1)*
EQW: BL/EP (% abnormal)	66/58	58/56	51/44	47/50	54/51	33/32	59/41	46/34
IG: Mean BL (overall)	133 (1)	80 (1)	2.7 (0.1)	1.2 (0.0)	2.1 (0.1)	4.8 (0.1)	31 (1)	5 (1)
IG: Week 26 Δ (overall)	-1 (1)	-1 (1)	0.0 (0.1)	0.0 (0.0)	-0.2 (0.1)*	0.0 (0.1)	-2 (1)*	-1 (1)
IG: Week 26 Δ (BL abnormal)	-5 (1)*	-3 (1)*	-0.2 (0.1)*	0.04 (0.02)*	-0.4 (0.2)*	-0.3 (0.1)*	-7 (2)*	-1 (2)
IG: BL/EP (% abnormal)	61/61	61/60	51/56	45/45	53/47	34/31	54/49	45/43

* p≤0.05 vs. baseline; BL = baseline; EP = endpoint; † Female/Male; TG = triglycerides; TC = total cholesterol; SBP = systolic blood pressure; DBP = diastolic blood pressure; Data are mean (SE).

Supported by: Amylin Pharmaceuticals, Inc. and Eli Lilly and Company



Supported by: Roche

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Effects of long term administration of miglitol and voglibose on plasma glucagon-like peptide-1 and gastric inhibitory polypeptide after a mixed meal ingestion

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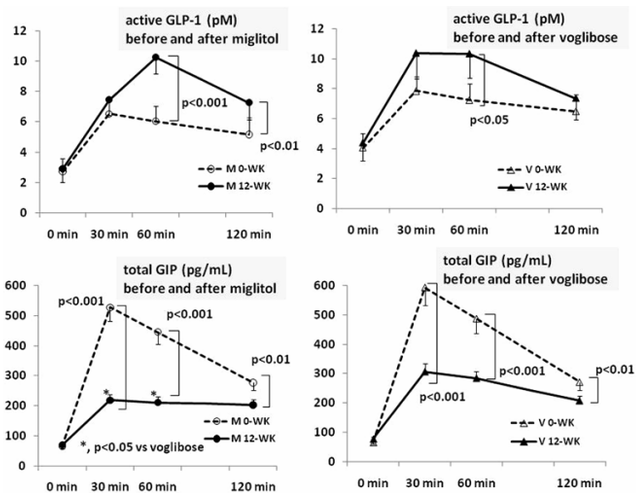
Background and aims: Recently, we have reported that a two-week administration of miglitol (M), an alpha-gucosidase inhibitor (AGI), induces prolonged and enhanced glucagon-like peptide-1 (GLP-1) and reduced gastric inhibitory polypeptide (GIP) responses after a mixed meal ingestion in Japanese type 2 diabetics (T2Ds). However, such effects of AGI on plasma incretins during long term or whether those effects of AGIs would be different according to types of AGIs (M, absorbed type; voglibose [V], non-absorbed type) have not been reported.
Materials and methods: In this multicenter, open 12-week trial, 26 and 24 Japanese T2Ds (age and HbA1c [mean]: 58.5, 59.5; 7.11 %, 7.08 %, respectively) with diet therapy and/or oral hypoglycemic agents other than AGIs were randomly assigned to receive 50 mg of M or 0.3 mg of V three times per day, respectively. We measured plasma glucose (PG), insulin (IRI), active GLP-1 and total GIP 0, 30, 60, 120 min after ingestion of a mixed meal (meal tolerance test [MTT]) at baseline and 12 weeks after administration of M or V. Active GLP-1 and total GIP levels were measured using commercially available ELISA kits (Linco Research, St Charles, MO, USA). Data are expressed as mean (SE).
Results: Baseline values of PG, IRI, GLP-1 and GIP during MTT were similar in both groups. PGs (mg/dL) at 30 and 60 min during MTT were significantly decreased both after M and V (at 30 min, 204.6 (6.4) vs. 160.0 (4.8) [M, p<0.001] and 208.6 (10.1) vs. 178.8 (7.4) [V, p<0.001]; at 60 min, 247.0 (8.6) vs. 184.2 (5.4) [M, p<0.001] and 246.0 (10.4) vs. 197.2 (7.9) [V, p<0.001]). PG at 30 min during MTT after M was significantly lower than that after V (p<0.05). IRIs at 30 and 60 min during MTT were also significantly decreased both after M and V. GLP-1 values at 60 and 120 min during MTT were significantly increased after M whereas only at 60 min value was significantly increased after V (figure). AUC (area under the curve during MTT) of GLP-1 was significantly increased after M (p<0.01) despite of marginal increase after V (p=0.059). GIPs at 30, 60 and 120 min during MTT were significantly decreased both after M and V (figure). GIPs at 30 and 60 min during MTT after M were significantly lower than those after V (figure). AUC of GIP after M was significantly lower than that after V (p<0.05). During the 12-week treatment, BMI reduction was significant after M (from 25.1 [1.0] to 24.7 [1.0], p<0.01) but not after V (from 25.6 [1.0] to 25.5 [1.0]).
Conclusions: Long term AGI administration induces increased GLP-1 and decreased GIP responses after a mixed meal ingestion in Japanese T2Ds. Absorbed type of M may have relatively strong effects modifying postprandial incretin responses compared with non-absorbed type of V. Slight but significant BMI reduction after M not but V might be attributable to these different effects on incretins.



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Improved glycaemic control with once-daily insulin detemir in combination with sitagliptin/metformin vs. sitagliptin/metformin ± sulphonylurea drugs

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Background and aims: Once-daily insulin detemir (IDet) provides stable, 24 hour glucose control and is often prescribed for patients with T2D as add-on to OADs. DPP-4 inhibitors, a new class of OADs, reduce degradation of GLP-1 mobilized in response to glucose intake, thereby improving endogenous glucose-dependent insulin secretion at mealtime. This study was a randomized, open-label, parallel group, 26-week trial designed to compare the efficacy and safety of two treatment regimens comprised of IDet in combination with the DPP-4 inhibitor sitagliptin (SITA) or SITA in combination with a subjects' prior sulphonylurea (SU) regimen, if any, and both groups continuing on metformin (Met), in insulin-naïve T2D patients who were poorly controlled by their previous regimens with Met ± other OADs.
Materials and methods: Treatment was for 26 weeks with once-daily IDet + SITA (100 mg QD) + Met (at pre-trial dose ≥1000 mg) (IDet/SITA; *n*=107) but with any prior SU discontinued, or with SITA + Met ± SU (continuing each subject's pretrial SU dosing, if any) (SITA ± SU; *n*=110). Both arms contained a similar percentage of subjects (75 and 77%, IDet/SITA vs. SITA, respectively) with pre-trial SU treatment experience.
Results: Observed baseline A1C in both arms was 8.5%. Estimated mean A1C reductions of 1.44 and 0.89% were achieved (IDet/SITA vs. SITA ± SU; est. mean diff. = 0.55%, 95% CI [-0.77, -0.33], $p<0.001$); estimated final mean A1c values were 7.08 and 7.64%, respectively; 45 vs. 24% of patients reached A1C ≤7% (Adjusted Odds Ratio (OR) = 3.20, 95% CI [1.65, 6.19], $p=0.001$) and 19 vs. 10% reached A1C ≤6.5% (OR 2.23, 95% CI [0.96, 5.20], $p=0.063$). Observed FPG baselines were 9.7 mmol/L (174.8 mg/dL) and 9.8 mmol/L (176.5 mg/dL), with final mean estimated FPG values 6.1 mmol/L (109.5 mg/dL) and 8.5 mmol/L (153.5 mg/dL), respectively (IDet/SITA vs. SITA ± SU; est. mean diff. = -2.45, 95% CI [-3.01, -1.88], $p<0.001$). After 26 weeks 9-point PG profiles were significantly lower for IDet/SITA at all time points except before dinner. No major hypoglycemia occurred in either arm. Rates for minor hypoglycemia (PG <3.1 mmol/L [56 mg/dL]) were low in both treatment arms, with no statistically significant differences between arms (0.47 vs. 0.48 episodes/patient-year, IDet/SITA vs. SITA ± SU; Adjusted Rate Ratio = 0.97, 95% CI: [0.35, 2.74]; p=NS). 36% of the subjects taking IDet/SITA achieved the HbA_{1c} ≤7% target without hypoglycemia in the last 3 months of treatment vs. 20% in the SITA ± SU arm (OR = 2.47, 95% CI [1.26; 4.81], $p=0.008$). Mean IDet dose rose from 0.11 to 0.59 U/kg during the 26 weeks' treatment. Body weight and BMI decreased in both arms (-0.81 vs. -1.66 kg and -0.30 vs. -0.58 kg/m^2 in IDet/SITA vs. SITA ± SU, respectively; p=NS). Data analysis of the subgroup who received SU pre-trial mirrored the data presented here for the full analysis set.
Conclusion: Glucose control was significantly more improved in patients on once-daily IDet/SITA vs. patients on SITA ± SU (Met in both arms) with

larger reductions in A1c and FPG occurring in the IDet/SITA arm. This was achieved with modest weight reductions and low hypoglycemia in both arms. These data support the use of once-daily IDet in combination with a DPP-4 inhibitor, SITA, and Met, with SU discontinued, as a safe and effective treatment option for insulin-naïve patients with T2D.
Supported by: Novo Nordisk

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Short-term effects of bed-time insulin versus GLP-1 analogue on resting energy expenditure in patients with type 2 diabetes
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Background and aims: GLP-1 analogues can be used instead of bed-time insulin therapy for poorly controlled type 2 diabetic (T2D) patients, with opposite influences on body weight. Beside the effect of GLP-1 on energy intake, it is not known whether changes of the Resting Energy Expenditure (REE) contribute to these distinct weight courses. We compared the early (first days) effects of both treatments on REE in T2D patients.
Materials and methods: Twenty-five T2D patients (8 women, 17men) poorly controlled despite maximal oral therapy, were included: 8 patients received GLP-1 analogue (exenatide® 5 µg twice-a-day); 17 received bed-time insulin analogue (Glargine® or Detemir®, initial dose 0.2UI/kg). REE was measured by indirect calorimetry before the first injection (Day0) and during two days (Day1 and 2) after initiating the treatments. Respiratory exchanges were monitored using a Sensor Medics Vmax 29N apparatus: VCO2 and VO2 were determined on 30 minutes intervals from 8 a.m. to 8h30 a.m., before breakfast, and REE was calculated according to Weir's equation. Body weight was assessed three months later.
Results: The two groups did not differ for age (GLP-1 group: 57±10 years; Insulin group: 56±10 years), gender, body weight (GLP-1: 90.7±10.0 kg; Insulin: 91.2±15.2 kg); HbA1c (GLP-1: 10.1±0.8%; Insulin: 9.4±1.3%), fasting plasma glucose level (GLP-1: 210 ± 85 mg/dl; Insulin: 210±46 mg/dl) and initial REE (Day0: GLP-1: 1821±240 kcal/24h; Insulin: 1883±363 kcal/24h). On insulin treatment, REE decreased by -3.5 % after the first injection (Day1:1816±386 kcal/24h, p=0.07 vs Day0) and by -5.8 % after the second injection (Day2: 1774±333 kcal/24h, p=0.03 vs Day0). REE was unchanged on GLP-1 analogue treatment (Day1:1842±255 kcal/24h, Day2:1798±240 kcal/24h, both NS vs Day0). Body weight increased after three months of insulin analogue therapy (M3: 92.2±15.2 kg vs Day0: 91.2 kg±15.6; p=0.08) whereas it decreased on GLP-1 analogue (M3:86.7±9.2kg vs 90.7±10.0; p=0.06), with significantly different body weight changes at three months (p=0.002).
Conclusion: REE decreases early after the introduction of insulin therapy, whereas it is not affected by the GLP-1 analogue. These different effects on REE probably contribute to the opposite weight changes with these treatments.

PS 79 SGLT-2 inhibitors

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Efficacy of dapagliflozin as monotherapy administered in the morning or evening to treat type 2 diabetes mellitus
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Background and aims: Dapagliflozin is a highly selective and reversible inhibitor of the renal sodium glucose co-transporter-2, being developed as an oral antidiabetic agent.
Materials and methods: We report here data from a 24-week randomized, double-blind, phase 3 trial of dapagliflozin administered in the morning or the evening to treatment-naïve type 2 diabetes mellitus patients (study ID: MB102013). Patients (n=485) with HbA1c 7.0-10% were randomized equally to receive placebo, or dapagliflozin 2.5, 5 or 10 mg, once-daily in the morning (main cohort) or evening (exploratory cohort). All patients received diet/exercise counselling. Efficacy measures included change from baseline in HbA1c, fasting plasma glucose (FPG) and body weight at week 24. Adverse events were assessed throughout the study. Patients were actively monitored for signs/symptoms suggestive of urinary tract infections (UTIs) and genital infections and MedDRA (Medical Dictionary for Regulatory Activities, version 11.1) preferred terms relating to these were prospectively defined.
Results: Reductions in mean HbA1c and FPG were seen in all treatment groups and were statistically significant in 5 and 10 mg dapagliflozin arms of the main cohort (Table). Mean body weight decreases were greater with all dapagliflozin doses than in placebo, although not reaching statistical significance. Efficacy with morning and evening dose of dapagliflozin was similar when compared to placebo. There were no clinically meaningful changes in serum electrolytes, creatinine, or cystatin-C in any treatment arm. Signs/symptoms/other reports suggestive of UTI and genital infection were more frequent with dapagliflozin than placebo (4.6-12.5% in dapagliflozin arms vs 4.0% with placebo for UTI; and 2.6-12.9% in dapagliflozin arms vs 1.3% with placebo for genital infection). The reported signs and symptoms of UTI and genital infection were resolved with standard care and rarely led to study discontinuation. There was no increase in hypoglycemia with dapagliflozin and hypoglycemic events were well balanced in all treatment arms including placebo. Overall rate of adverse events was similar between the morning and evening dosing groups. There were no reports of nocturia in the main morning cohort and 1, 2, and 3 patients with nocturia in dapagliflozin 2.5, 5 or 10 mg evening dose arms, respectively.
Conclusion: Dapagliflozin is equally efficacious with morning or evening dosing with no notable difference in the number or type of adverse events between the two cohorts. Treatment with dapagliflozin resulted in clinically meaningful decreases in HbA1c and FPG in type 2 diabetes mellitus patients along with a favorable effect on weight.

Table. Change from baseline at week 24 in HbA1c, FPG, and body weight*

		Dapagliflozin morning dose (main cohort)			Dapagliflozin evening dose (exploratory cohort)**		
	Placebo N=75	2.5mg N=65	5mg N=64	10mg N=70	2.5mg N=67	5mg N=68	10mg N=76
HbA_{1c} (%)	-0.23 (0.10)	-0.58 (0.11)	-0.77[a] (0.11)	-0.89[b] (0.11)	-0.83 (0.11)	-0.79 (0.11)	-0.79 (0.10)
FPG (mg/dL)	-4.1 (3.9)	-15.2 (4.2)	-24.1[a] (4.3)	-28.8[b] (4.0)	-25.6 (4.1)	-27.3 (4.2)	-29.6 (4.0)
Weight (kg)	-2.2 (0.4)	-3.3 (0.5)	-2.8 (0.5)	-3.2 (0.5)	-3.8 (0.5)	-3.6 (0.5)	-3.1 (0.4)

Data are mean (SE). *Mean value after adjusting for baseline value with last observation carried forward. **Per study design, the exploratory cohort was not statistically tested. [a]$P<0.001$; [b]$P<0.0001$

Supported by: Bristol-Myers Squibb and AstraZeneca

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Dapagliflozin: an effective treatment option in patients with type 2 diabetes across stages of disease

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Background and aims: Dapagliflozin, a selective inhibitor of the renal sodium glucose co-transporter 2 (SGLT2), helps lower excess glucose levels in an insulin-independent manner by increasing urinary glucose excretion. We analysed data from 3 double-blind, randomised, placebo-controlled trials of dapagliflozin in patients with inadequate glycaemic control at different stages of type 2 diabetes (T2DM), as reflected by their treatment regimens.
Materials and methods: Patients with T2DM were treated with dapagliflozin 2.5, 5 or 10 mg or placebo as monotherapy (study MB102013; N = 485 [main cohort N = 274]), as an add-on to metformin (MB102014; N = 546) and as an add-on to insulin with or without up to 2 oral anti-diabetic drugs (AZ006; N = 807). The primary end point for all trials was change in HbA1c at Week 24.
Results: Significant improvements in the glycaemic measures of HbA1c and fasting plasma glucose (FPG) were observed with dapagliflozin (Table) regardless of T2DM stage and background medication and with no increase in major hypoglycaemia. The need for rescue therapy with anti-diabetic agents (monotherapy and metformin studies) or insulin for failing to achieve prespecified glycaemic targets was reduced with dapagliflozin. Weight loss was seen in all studies, which reached statistical significance for patients in the metformin and insulin studies in dapagliflozin treatment groups compared to placebo. Adverse events (AE), serious AEs and study discontinuations were similar across all groups, although active solicitation revealed increased reports of signs, symptoms and events suggestive of genital infection in dapagliflozin groups. There were increased reports suggestive of urinary tract infection with dapagliflozin in the monotherapy and insulin trials but not in the metformin trial.
Conclusion: Dapagliflozin produced significant improvement in glycaemic control in patients at various stages in the progression of T2DM, from treatment-naïve to those on insulin with or without oral anti-diabetic agents. Dapagliflozin, due to its insulin-independent mechanism, is a potential therapy to improve glycaemic control and body weight in patients with T2DM across stages of disease.

	MON		MET		INS	
	PLA	DAPA 10 mg	PLA + MET	DAPA 10 mg + MET	PLA + INS	DAPA 10 mg + INS
n	75	70	137	135	193	194
HbA1c[a] (%) ± SE	-0.23± 0.10	-0.89[b]± 0.11	-0.30± 0.07	-0.84[b]± 0.07	-0.30± 0.05	-0.90[b]± 0.05
FPG[a] (mg/dL) ± SE	-4.1± 4.0	-28.8[c]± 4.3	-6.0± 2.7	-23.5[c]± 2.7	3.3± 3.4	-21.7[c]± 3.3
Body Weight[a] (kg) ± SE	-2.2± 0.4	-3.2± 0.5	-0.9± 0.2	-2.9[c]± 0.2	0.02± 0.2	-1.7[c]± 0.2
Patients (%) reaching HbA1c Goal <7.0%, Week 24	32	51	26	41[c]	9	22[d]
Patients rescued or discontinued due to hyperglycemia (%), Week 24[e]	12	0	15	4	29	10

10 mg data shown for presentation
[a]Adjusted mean change from baseline to Week 24 (LOCF)
[b]Primary endpoint, $P \leq .0001$ vs PLA
[c]Statistically significant based on sequential testing for secondary end points at $\alpha = 0.05$
[d]*Nominal P* = 0.0003 vs PLA + INS
[e]Adjusted percent of patients rescued with anti-diabetic agents (monotherapy and metformin studies) or insulin uptitration (insulin study), or discontinued, based on pre-specified glycaemic targets.

Supported by: Bristol-Myers Squibb and AstraZeneca

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Efficacy and safety of dapagliflozin in patients with type 2 diabetes mellitus and inadequate glycaemic control on glimepiride monotherapy

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Background and aims: In management of patients with type 2 diabetes mellitus (T2DM), progressive deterioration of glycaemic control is commonly observed, requiring treatment intensification with additional drug therapy. Dapagliflozin (DAPA), a selective inhibitor of sodium-glucose co-transporter 2 that inhibits renal glucose reabsorption, has been shown to reduce hyperglycaemia and body weight in patients with T2DM without a significantly increased risk of hypoglycaemia. We assessed efficacy, safety and tolerability of DAPA as add-on to glimepiride in patients with uncontrolled T2DM.
Materials and methods: This 24-week (wk) randomized, double-blind, placebo-controlled, parallel-group multicentre trial (D1690C00005) in Europe/Asia-Pacific enrolled patients with T2DM uncontrolled on at least half the maximum recommended dose of sulfonylurea alone (HbA1c 7.0-10.0%). A total of 597 adult subjects were randomized to DAPA (2.5, 5, or 10 mg/d) or placebo (PLA) added to open-label glimepiride 4 mg/d for 24 wk. Primary endpoint was change from baseline in HbA1c at 24 wk. Secondary endpoints included changes in other glycaemic parameters and body weight. Interim safety data are also described.
Results: At 24 wk, DAPA showed dose-dependent significant reductions in HbA1c from baseline vs PLA (Table). DAPA showed weight loss relative to PLA at wk 24 (-0.46, -0.84, -1.54 kg for DAPA 2.5, 5,10 mg, respectively) which was statistically significant for DAPA 5 and 10 mg treatment groups. DAPA 5 and 10 mg caused significant improvements in oral glucose tolerance test (OGTT) response and in fasting plasma glucose (FPG) at wk 24. Compared with PLA, significantly more DAPA 5 and 10 mg-treated patients achieved HbA1c <7.0% at wk 24. Adverse events (AEs) were reported in 47%, 52%, 48%, and 50% of patients receiving PLA and DAPA 2.5, 5, 10 mg, respectively; frequency of drug-related AEs was similar across all treatment groups. Actively solicited events suggestive of urinary tract infections were 6.2%, 3.9%, 6.9% and 5.3% in PLA and DAPA 2.5, 5, 10 mg, respectively. No AE of kidney infection was reported. Actively solicited events suggestive of genital infections were 0.7%, 3.9%, 6.2% and 6.6% in PLA and DAPA 2.5, 5, 10 mg, respectively. No cases of genital tract infection led to treatment discontinuation. Hypoglycaemic events were 4.8%, 7.1%, 6.9% and 7.9% in PLA and DAPA 2.5, 5, 10 mg groups, respectively. No clinically meaningful mean changes from baseline in serum creatinine or electrolytes were observed. Adjusted mean changes from baseline at wk 24 in seated systolic/diastolic blood pressure were -1.2/-1.4, -4.7/-1.1, -4.0/-1.7 and -5.0/-2.8 mmHg for PLA, and DAPA 2.5, 5,10 mg, respectively.
Conclusions: DAPA added to glimepiride in T2DM patients uncontrolled with sulphonylurea monotherapy significantly improves HbA1c, reduces weight and is well tolerated.

	PLA + glim [n=145]	DAPA 2.5 mg + glim [n=154]	DAPA 5 mg + glim [n=142]	DAPA 10 mg + glim [n=151]
HbA1c, baseline (%), mean (SD)	8.15 (0.74)	8.11 (0.75)	8.12 (0.78)	8.07 (0.79)
Week 24 (LOCF) absolute change from baseline in HbA1c (%), adjusted mean (SE)	-0.13 (0.063)	-0.58 (0.060)#	-0.63 (0.063)#	-0.82 (0.061)#
Body weight, baseline (kg), mean (SD)	80.9 (15.8)	81.9 (19.0)	81.0 (18.6)	80.6 (17.9)
Week 24 (LOCF) absolute change from baseline in body weight (kg), adjusted mean (SE)	-0.72 (0.23)	-1.18 (0.22)	-1.56 (0.23)†	-2.26 (0.22)#
OGTT, baseline (mg/dL)*, mean (SD)	158.6 (58.75)	140.4 (68.18)	151.2 (64.21)	157.3 (69.03)
Week 24 (LOCF) change from baseline in OGTT (mg/dL)*, adjusted mean (SE)	-6.0 (5.02)	-37.5 (4.68)	-32.0 (4.84)†	-34.9 (4.56)#
Week 24 (LOCF) adjusted proportion (%) of patients with HbA1c <7%, (95% CI)	13.0 (7.6, 18.4)	26.8 (20.2, 33.3)	30.3 (23.4, 37.1)†	31.7 (24.7, 38.7)#

*measured 2 h after ingestion of 75 g glucose
#p<0.0001, †p<0.01 vs PLA
Primary endpoint tested at alpha=0.019 applying Dunnett adjustment; secondary endpoints tested following a sequential procedure at alpha=0.05

Supported by: AZ and BMS

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Effect of dapagliflozin, a novel insulin-independent treatment, over 48 weeks in patients with type 2 diabetes poorly controlled with insulin

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Background and aims: Escalating insulin doses to achieve glycaemic targets in type 2 diabetes mellitus (T2DM) increases risk of weight gain, fluid retention and hypoglycaemia, often presenting a therapeutic dilemma. Dapagliflozin, a selective inhibitor of sodium glucose co-transporter 2 (SGLT2), reduces glucose levels in T2DM patients in an insulin-independent manner by inducing urinary glucose excretion. Resulting caloric loss may also help prevent weight gain in insulin-treated patients.
Materials and methods: To determine efficacy and safety of dapagliflozin in T2DM poorly controlled with insulin, patients were randomised to placebo, 2.5, 5, or 10 mg dapagliflozin added to unchanged background insulin therapy with or without concomitant oral antidiabetic drugs (Trial D1690C00006). Primary outcome measure was change from baseline in HbA1c at week (wk) 24 (LOCF). Patients completing the 24-wk primary efficacy phase continued in a 24-wk site- and subject-blinded extension phase.
Results: A total of 808 patients were randomised. Mean baseline values were: HbA1c, 8.5%; fasting plasma glucose (FPG), 178 mg/dL; body weight (BW), 94 kg; insulin dose, 77 IU/day. Of 711 patients who completed the primary efficacy phase, 676 completed 48 wks of double-blind treatment. Significant reductions in HbA1c, FPG, BW and insulin dose were observed with dapagliflozin relative to placebo in primary efficacy phase with no clinically relevant increase in hypoglycaemia. Reductions in glycemic parameters and BW were sustained over 48 wks. Insulin requirements over the 48 wks continued to increase with placebo but not in dapagliflozin groups. Overall, adverse events (AEs), serious AEs, and study discontinuations were comparable across groups. Active solicitation revealed increased reports of signs and symptoms suggestive of urinary (7.9-10.8% vs 5.1% for placebo) and genital (6.4-10.7% vs 2.5% for placebo) tract infection with dapagliflozin, most of which occurred during first 24 wks, were generally mild and responded to routine management. Two cases of pyelonephritis (1 each in 2.5 and 5 mg groups) responded to antibiotic therapy and did not lead to discontinuation. There were no discontinuations due to hypoglycaemia. There was a trend of small mean decreases in systolic blood pressure, without increased frequency of orthostatic hypotension, in dapagliflozin groups.
Conclusion: Once-daily oral dapagliflozin improved glycaemic control without an increase in daily insulin requirements and led to weight loss in patients with T2DM poorly controlled with insulin over a 48-wk treatment period. Attenuating the cycle of escalating insulin dose and weight gain represents an improvement for this patient population.

		Dapagliflozin dose + INS		
Adjusted mean change from baseline (SE) at Week 24	PBO + INS (n = 193)[d]	2.5 mg (n = 202)[d]	5 mg (n = 211)[d]	10 mg (n = 194)[d]
HbA1c (%)[e]	-0.30 (0.05)	-0.75 (0.05)[a]	-0.82 (0.05)[a]	-0.90 (0.05)[a]
Body weight (kg)[e]	0.02 (0.18)	-0.98 (0.18)[a]	-0.98 (0.17)[a]	-1.67 (0.18)[a]
insulin dose (IU/d)[f]	5.08 (0.94)	-1.80 (0.92)[a]	-0.61 (0.90)[a]	-1.16 (0.94)[a]
FPG (mg/dL)[e]	3.3 (3.4)	-12.5 (3.2)[b]	-18.8 (3.1)	-21.7 (3.3)[a]
Mean change from baseline (SE) at Week 48[c]				
HbA1c (%)[e]	-0.43 (0.07)	-0.74 (0.06)	-0.94 (0.06)	-0.93 (0.06)
Body weight (kg)[e]	-0.18 (0.30)	-1.11 (0.26)	-1.21 (0.25)	-1.79 (0.26)
Total daily insulin dose (IU/d)[f]	10.54 (1.49)	-0.92 (1.43)	0.30 (1.41)	-0.70 (1.44)
FPG (mg/dL)[e]	-4.4 (4.1)	-17.0 (3.4)	-20.8 (3.3)	-21.5 (3.4)
		Dapagliflozin dose + INS		
	PBO + INS (n =197)[g]	2.5 mg (n =202)[g]	5 mg (n =212)[g]	10 mg (n =196)[g]
Seated systolic blood pressure (mmHg) mean change from baseline (SE) at Week 48	-0.2 (1.3)	-5.4 (1.2)	-3.8 (1.1)	-5.2 (1.1)
Seated diastolic blood pressure (mmHg) mean change from baseline (SE) at Week 48	-1.3 (0.7)	-2.3 (0.7)	-3.1 (0.6)	-2.9 (0.7)
Major hypoglycemia (n [%]) over 48 weeks	2 (1.0)	3 (1.5)	2 (0.9)	3 (1.5)
Total hypoglycemia (n [%]) over 48 weeks	102 (51.8)	122 (60.4)	118 (55.7)	105 (53.6)

P value (vs PBO + INS): [a]≤0.0001; [b]0.0008 [c]p-values were not calculated for the extension phase
[d] Randomized patients who took at least one dose of study medication and who have baseline and at least one post-baseline efficacy assessment
[e] Excluding data after insulin up-titration
[f] Including data after insulin up-titration
[g] Randomized patients who took at least one dose of study medication

Supported by: AstraZeneca and Bristol-Myers Squibb

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Dapagliflozin lowered rate of insulin uptitration/study discontinuation from lack of glycaemic control in 48-week study of type 2 diabetes patients poorly controlled on insulin therapy

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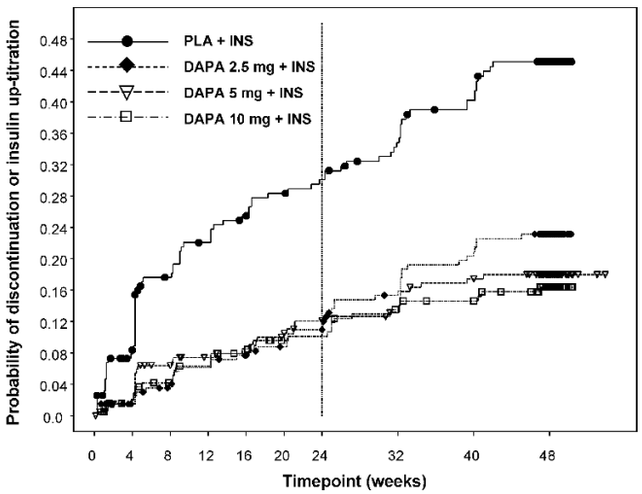
Background and aims: In patients with poorly controlled type 2 diabetes (T2DM) receiving insulin (INS), the clinical utility of escalating INS doses

to achieve glycaemic control is often limited by associated weight gain, fluid retention, hypoglycaemia and patient acceptance. Dapagliflozin (DAPA), a selective inhibitor of sodium-glucose cotransporter 2 in development for T2DM treatment, increases renal excretion of glucose and lowers blood glucose via an INS-independent mechanism. The coadministration of DAPA with INS can potentially attenuate escalating INS doses and associated side effects.

Materials and methods: Patients with poorly controlled T2DM (n=808; mean baseline HbA1c 8.53%) were randomized to placebo (PLA), 2.5, 5, or 10 mg/d DAPA in addition to background INS (mean baseline dose 77 IU/d) ± other oral antidiabetic drugs. Primary glycaemic and safety endpoints have been reported separately; here we report time to study discontinuation or INS up-titration due to lack of glycaemic control (DISC-UP) for up to 48 weeks (wk) of continued centre- and patient-blinded treatment. INS was up-titrated if HbA1c was >8% or fasting plasma glucose was >9.9mmol/L from 24-48 wk. Weight gain, peripheral oedema and DISC due to hypoglycaemia and other causes were also assessed.

Results: Time to reach DISC-UP was substantially prolonged in all DAPA + INS groups vs PLA + INS (figure). At 48 wk, the proportion of patients with DISC-UP was 42.8% in the PLA + INS group vs 21.7%, 15.6% and 15.3% in the DAPA + INS groups, with the greatest difference seen between the DAPA 10 mg + INS vs PLA + INS groups (−27.5%; 95% CI −35.9 to −19.1%). Patients receiving DAPA maintained glycaemic control and sustained reductions in body weight from 24-48 wk compared with placebo. At 24 wk, the proportion of patients with weight loss >3% was 10.4% in the PLA + INS group vs 17.7-23.3% in the DAPA + INS groups. Corresponding values at 48 wk were 10.4% and 21.6-24.5%, respectively. The frequency of peripheral oedema was 7.6% in the PLA + INS group vs 4%, 2.4% and 4.6% in the DAPA + INS groups. The frequency of DISC due to adverse events (AEs) in the PLA + INS group was 4.6% vs 3.5%, 7.1%, and 5.1% in the DAPA + INS groups, respectively. Corresponding values for DISC due to serious AEs were 1.5%, 1%, 2.4% and 2.6%, respectively. There were no DISCs due to hypoglycaemia.

Conclusion: Over 48 wk, DAPA treated patients were less likely to require DISC-UP due to poor glycaemic control. This insulin sparing effect of DAPA was further demonstrated by an increased frequency of weight loss and a reduced frequency of peripheral oedema over time.



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Canagliflozin, an inhibitor of sodium glucose co-transporter 2, improves glycaemic control, lowers body weight, and improves beta cell function in subjects with type 2 diabetes on background metformin

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Background and aims: Inhibition of sodium glucose co-transporter 2 (SGLT2) is being studied as a novel modality of treatment for type 2 diabetes mellitus (T2DM). We therefore sought to evaluate the safety, tolerability, and efficacy of canagliflozin (CANA, JNJ-28431754/TA-7284), a potent inhibitor of SGLT2, in subjects with T2DM with inadequate glycemic control on background metformin therapy. Since loss of beta cell function (BCF) underlies the progressive deterioration of glycemic control in T2DM, we also assessed the effect of CANA on a measure of BCF.

Material and methods: In a double-blind, placebo (PBO)-controlled, dose-ranging study, subjects (N=451) were randomized to PBO; CANA 50, 100, 200, 300 mg once daily (OD); 300 mg BID; or sitagliptin (SITA) 100 mg OD for 12 weeks. BCF was assessed using HOMA2-B% calculated from plasma glucose (PG) and C-peptide concentrations at week 12.

Results: Mean baseline characteristics (age 53 years, HbA_{1c} 7.7%, fasting plasma glucose [FPG] 9.0 mmol/l, BMI 31.5 kg/m^2) were similar across treatment groups. A significant increase in urinary glucose (UG)/creatinine in all CANA doses was observed. At week 12, the reductions from baseline in FPG and HbA_{1C} were statistically significant for all CANA arms and for SITA compared with PBO, with maximal/similar decreases at CANA 300 mg OD and BID doses (Table). Significant, dose-related weight reductions vs PBO were seen across all CANA arms but not with SITA (Table). Significant improvements in HOMA-2B% were seen in subjects treated with 100 mg of CANA and greater (Table). In general, adverse events (AEs) were transient, mild to moderate in intensity, and balanced across arms except for an increase in symptomatic genital infections that were non dose-dependent: 3-8% in CANA arms, 2% in PBO, and 2% in SITA. Similar incidences of discontinuations due to AEs and serious AEs across arms were observed. Urinary tract infections were reported in 3-9% of CANA arms, without dose-dependency, 6% of PBO, and 2% of SITA. Hypoglycemia was reported in 0-6% of CANA arms, without dose-dependency, 2% of PBO, and 5% of SITA. In CANA arms, after 12 weeks of treatment, no safety signals in laboratory studies, ECG, or vital signs were observed.

Conclusion: In subjects with T2DM with mild to moderate hyperglycemia on metformin background therapy, adding CANA was generally well tolerated, provided clinically meaningful HbA_{1c} reductions, reduced body weight, and led to suggestive improvements in BCF.

Mean Value (SD)	PBO n=65	50 mg OD n=64	100 mg OD n=64	200 mg OD n=65	300 mg OD n=64	300 mg BID n=64	SITA n=65
Baseline HbA_{1c}, %	7.7 (0.83)	8.0 (1.01)	7.8 (0.97)	7.6 (0.79)	7.7 (1.04)	7.7 (0.88)	7.6 (0.95)
Final HbA_{1c}, %	7.5 (0.96)	7.2 (0.88)	7.1 (0.85)	6.9 (0.68)	6.8 (0.82)	6.8 (0.72)	6.9 (0.92)
? HbA_{1c}, %		-0.45[a]	-0.51[a]	-0.54[a]	-0.71[a]	-0.73[a]	-0.56[a]
Baseline FPG, mmol/l	9.0 (2.10)	9.5 (2.49)	9.3 (2.30)	8.8 (2.04)	8.8 (2.38)	8.6 (1.77)	8.8 (2.31)
? FPG, mmol/l		-0.9[a]	-1.4[a]	-1.8[a]	-1.8[a]	-1.7[a]	-1.0[a]
Baseline weight, kg	85.5 (19.58)	87.5 (16.40)	87.7 (15.49)	87.7 (17.22)	87.8 (15.79)	86.3 (19.90)	87.0 (18.00)
? Weight, %		-1.3[b]	-1.5[b]	-1.6[a]	-2.3[a]	-2.3[a]	0.4
Baseline overnight UG/creatinine, mg/mg	5.7 (12.52)	10.1 (17.99)	4.8 (9.02)	4.8 (9.61)	8.2 (26.66)	6.2 (11.89)	6.1 (14.84)
? Overnight UG/creatinine, mg/mg		36.1[a]	49.3[a]	48.2[a]	49.0[a]	60.3[a]	-3.3
Baseline HOMA2-B,%	54.4 (21.12)	51.5 (24.52)	51.8 (23.63)	58.8 (33.88)	56.4 (25.47)	65.0 (31.27)	67.7 (43.17)
? HOMA2-B,%		3.8	12.0[b]	15.9[a]	18.0[a]	17.7[a]	13.2[b]

? = Placebo-adjusted least squares mean changes from baseline [a]p 0.001, [b]p=0.01.

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Canagliflozin, a novel inhibitor of sodium glucose co-transporter 2, increases 24-hour urinary glucose excretion and reduces body weight in obese subjects over 2 weeks of treatment

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Background and aims: Sodium glucose co-transporter 2 (SGLT2) inhibition is considered a promising new approach for the treatment of patients with type 2 diabetes. This phase 1 study aimed to assess the pharmacodynamics and safety of canagliflozin (CANA; JNJ-28431754/TA-7284), an inhibitor of SGLT2, in obese subjects, including some with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT).

Materials and methods: In a double-blind, ascending, multiple-dose inpatient study, 80 obese subjects (40 women) were randomized to CANA (30, 100, 300, or 600 mg daily [OD] or 300 mg BID) or placebo (PBO) for 14 days. Subjects followed a fixed weight-maintaining diet 15 days prior to dosing until the end of the study. IFG (5.5 mmol/l ≤ fasting plasma glucose [FPG] < 6.93 mmol/l) and/or IGT (plasma glucose [PG] 2 h after a morning meal of

7.7-10.95 mmol/l) was present in 31 subjects. Renal threshold for glucose excretion (RT_G) was calculated from urinary glucose excretion (UGE), glomerular filtration rate, and PG.

Results: At baseline, subjects had a median age of 43 years (range, 21-59), a BMI of 33 kg/m^2 (range, 30-39), and an FPG of 5.12 mmol/l (range, 3.14-7.26). CANA significantly increased 24-h UGE (UGE_{24h}) on days 1-14 (Table), but there were no significant changes in FPG, mean 24-h PG, or insulin levels. Body weight decreased over the 14 days. Self-reported appetite and satiety measures did not change significantly.

	PBO	CANA				
Mean (SD)	n=20	30 mg OD n=12	100 mg OD n=12	300 mg OD n=12	600 mg OD n=12	300 mg BID n=12
UGE_{24h} day -1, g	<0.1 (0.05)	0.2 (0.3)	0.2 (0.6)	<0.1 (0.02)	<0.1 (0.02)	0.1 (0.1)
UGE_{24h} day 14, g	<0.1 (0.04)	9 (4.2)	33[a] (9.7)	47[a] (23)	50[a] (13)	61[a] (16)
Body weight day -1, kg	89.9 (12.8)	83.2 (12.9)	90.1 (10.0)	85.1 (9.6)	90.5 (16.3)	98.0 (13.3)
ΔBody weight day 14 vs day -1, kg	-1.4 (1.1)	-2.9[b] (1.5)	-2.7[b] (0.9)	-2.1 (1.6)	-3.4[a] (1.3)	-3.5[a] (1.4)

[a]p<0.0001; [b]p<0.01.

CANA decreased RT_G in a dose-dependent manner, with maximal effect on lowering of the RT_G to 3.5±0.9 mmol/l. In subjects with IFG and /or IGT on day -1, (n=31), treatment with CANA (n=22, all doses pooled) produced a significant reduction in 24-h mean PG (MPG) from 6.1±0.52 mmol/l on day -1 to 5.6 ±0.48 mmol/l on day 14 (p<0.05) compared with a corresponding change from 6.1±0.66 mmol/l to 5.9±0.77 mmol/l (p>0.05) after PBO. CANA was generally well tolerated, with no hypoglycemia. Adverse events were transient and mild to moderate in severity. The most frequently reported treatment-emergent adverse events were mild gastrointestinal disorders (20% in PBO and 0-50% across CANA dose groups). There were no clinically meaningful changes in urine volume or frequency, vital signs, ECGs, or laboratory tests.

Conclusion: CANA was well tolerated and increased 24-h UGE, decreased RT_G, and reduced body weight in obese healthy subjects. In addition, the data suggest that CANA is effective in reducing MPG in patients with IFG and/or IGT.

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Canagliflozin lowers the renal threshold for glucose excretion in lean, obese and type 2 diabetic subjects

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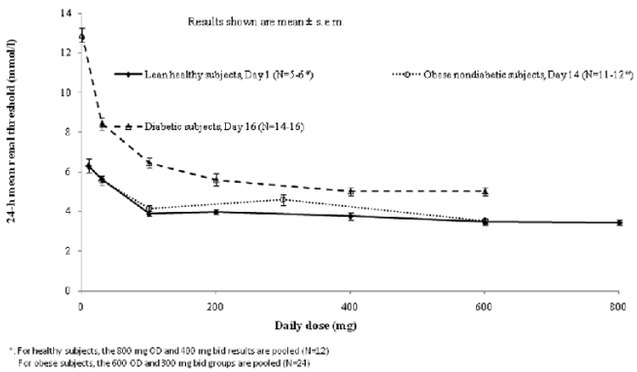
Background and aims: Urinary glucose excretion (UGE) can be approximated by a threshold relationship in which almost no glucose is excreted when plasma glucose (PG) concentrations are below the renal threshold for glucose excretion (RT_G), and the rate of UGE increases with PG when PG exceeds RT_G. The value of RT_G is dependent on the capacity of renal glucose transporters; pharmacological inhibitors of the transporters reduce RT_G. We aimed to determine the effects of canagliflozin (CANA; JNJ-28431754/TA-7284), an inhibitor of sodium glucose co-transporter 2 (SGLT2), on RT_G in healthy lean and obese subjects and subjects with type 2 diabetes (T2DM).

Materials and methods: Data from 3 clinical studies assessing the safety, tolerability, and pharmacodynamics of CANA were used. In study 1, lean males (BMI=20-30 kg/m^2) were treated with 1 dose of 10-800 mg or 400 mg BID CANA (n=6/group). In study 2, obese otherwise healthy subjects (BMI=30-39 kg/m^2) were treated with 30-600 mg once daily (OD) or 300 mg BID CANA (n=12/group) for 2 weeks. In study 3, subjects with T2DM were treated with 30-400 mg OD or 300 mg BID CANA (n=14-16/group) for 2 weeks. 24-h PG profiles, creatinine clearance (to estimate GFR), and UGE over 6 time intervals were measured. RT_G was determined from these measurements by assuming a threshold relationship between UGE and PG and determining the value of RT_G so that UGE calculated from the threshold approximation equals the measured UGE. This approach extends methods used for renal thresholds of other analytes by accounting for the dynamic changes in PG. 24-h mean RT_G (MRT_G) was calculated from RT_G values in each interval.

Results: CANA was well tolerated in the 3 studies with no observed hypoglycemia. Adverse events were transient and mild to moderate in severity. Urine volume, electrolyte excretion, renal function, and lab safety values did not change meaningfully. The method for determining RT_G provided highly reproducible results in placebo-treated T2DM subjects (within-subject CV <3%). CANA dose-dependently reduced RT_G; maximally effective doses (>200 mg OD in lean, >300 mg OD obese) reduced MRT_G to 3.5±0.4 mmol/l (mean±SD) in lean and 3.5±0.6 mmol/l in obese subjects (Figure). In subjects with T2DM, MRT_G was elevated before CANA (13.8±1.6 mmol/l vs commonly reported values of 10-11 mmol/l in healthy subjects), consistent with reports of increased renal glucose reabsorption in T2DM, and CANA dose-dependently reduced MRT_G to a minimum of 5.2±0.9 mmol/l at doses 200 mg. Maximally effective doses maintained maximal suppression of RT_G for the full 24-h period; with lower doses, RT_G rose from the nadir in later intervals.

Conclusion: RT_G in T2DM subjects before treatment was higher than often quoted values of 10-11 mmol/l. CANA dose-dependently decreased RT_G in all 3 studies and values in subjects with T2DM were modestly higher than nondiabetic subjects given the same doses. CANA reduces RT_G in T2DM subjects to ~5 mmol/l, a level not predicted to increase the risk of hypoglycemia.



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Canagliflozin, a novel inhibitor of sodium glucose co-transporter 2, improved glucose control in subjects with type 2 diabetes: Results of a phase 1b study

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Background and aims: Inhibition of renal sodium glucose co-transporter 2 (SGLT2) is a promising new approach for treating type 2 diabetes mellitus (T2DM). This study evaluated the safety, tolerability, and pharmacodynamic effects of canagliflozin (CANA, JNJ-28431754/TA-7284), a novel SGLT2 inhibitor, in subjects with T2DM. The effects of CANA on urinary glucose excretion (UGE), renal threshold for glucose excretion (RT_G), plasma glucose (PG), body weight (BW), and beta cell function (BCF) were assessed.

Materials and methods: In this double-blind, multiple-dose study, subjects with T2DM discontinued their anti-hyperglycemic medications for 2 weeks and were then randomized to 2 weeks of treatment with CANA 30, 100, 200, 400 mg daily (OD) or 300 mg BID, or placebo (PBO) while domiciled at study centers and maintaining an isocaloric diet. Subjects were domiciled from day -2 before treatment until 4 days after final treatment. At pretreatment baseline (day -1) and at day 16, UGE, PG, fasting plasma glucose (FPG), C-peptide, and RT_G (calculated from UGE, PG, and GFR) were measured. BCF was assessed using the calculated insulin secretion rate (ISR) at 10 mM PG, determined from a model-based method using the frequently measured PG and C-peptide concentrations.

Results: 97 subjects (70 M/27 F; mean age 53 years, weight 91.8 kg, BMI 30.88 kg/m^2, HbA_{1C} 8%) were randomized to CANA (5 doses) or PBO. CANA treatment increased UGE and decreased RT_G in a dose-dependent manner (Table). CANA reduced 24-h PG, FPG, and BW (by approximately 1-1.5 kg >PBO). BCF also improved in subjects treated with ≥100 mg of CANA

(Table). Adverse events were transient, mild to moderate in intensity, and balanced across treatment groups; 1 episode of vaginal candidiasis occurred in a CANA-treated subject. CANA treatment did not cause hypoglycemia, consistent with the RT_G with CANA remaining above a hypoglycemic threshold. There were no clinically meaningful changes in urine volume, electrolyte excretion, renal function, or routine lab safety test values.

Conclusion: In subjects with T2DM, CANA lowered RT_G, lowered PG concentrations without causing hypoglycemia, reduced BW, and also improved BCF. In this study, CANA was well tolerated. If this profile is demonstrated in further studies, CANA could be a useful choice for treating T2DM.

Mean Parameter	PBO n=19	30 mg n=16	100 mg n=16	200 mg n=16	400 mg n=16	300 mg BID n=14
24-h UGE day -1, g (SD)	24.5 (31.9)	10.9 (11.9)	11.5 (11.5)	22.2 (32.9)	43.7 (67.9)	12.5 (16.4)
Δ24-h UGE day -1 to 16, g (SD)	-9.71 (19.1)	69.1[a] (31.2)	76.4[a] (28.1)	88.0[a] (35.4)	113[a] (29.0)	88.3[a] (36.5)
24-h RT_G day -1, mmol/l (SD)	13.4 (1.4)	14.4 (1.5)	13.5 (1.4)	13.7 (1.9)	14.5 (1.9)	13.3 (1.6)
24-h RT_G day 16, mmol/l (SD)	12.9 (1.4)	8.4[a] (1.3)	6.5[a] (1.0)	5.6[a] (1.2)	5.0[a] (0.8)	5.0[a] (0.7)
24-h PG on day -1, mmol/l (SD)	12.4 (2.8)	12.4 (1.9)	11.8 (2.3)	12.3 (2.9)	13.4 (3.9)	11.4 (2.9)
Δ24-h PG day -1 to 16, mmol/l (SD)	-1 (1.4)	-1.2 (1.0)	-2.6[a] (1.4)	-3.2[a] (2.4)	-3.6[a] (2.1)	-3.0[a] (1.7)
FPG day -1, mmol/l (SD)	11.7 (2.4)	11.1 (1.7)	11.4 (2.0)	11.6 (2.8)	11.6 (3.0)	11.1 (2.5)
ΔFPG day-1 to 16, mmol/l (SD)	-1.4 (2.0)	-1.2 (1.2)	-3.0[a] (1.3)	-3.6[a] (2.9)	-3.3[a] (2.7)	-3.7[a] (2.0)
ISR at 10 mmol/l glucose, day -1, pmol/min/m^2 (SD)	135 (76)	139 (59)	160 (89)	155 (104)	145 (71)	181 (84)
ΔISR at 10 mmol/l glucose, day -1 to 16, pmol/min/m^2 (SD)	24 (40)	20 (36)	94[b] (32)	86[b] (66)	94[b] (74)	120[b] (86)

[a]$p<0.01$; [b]$p\leq0.05$; vs placebo.

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The potent and highly selective sodium-glucose co-cransporter (SGLT-2) inhibitor BI 10773 is safe and efficacious as monotherapy in patients with type 2 diabetes mellitus

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Background and aims: The aim of this randomized double-blind, placebo (PBO)-controlled, parallel-group comparison study was to investigate the efficacy and safety of BI 10773 for 12 weeks in patients with T2DM and insufficient glycemic control.

Materials and methods: A total of 408 patients (baseline means [standard deviation]: HbA_{1c} 7.9 [0.8]%, age 57.5 [9.8] years, body mass index 29.0 [4.6] kg/m^2) who were treatment naive or had undergone a 4-week washout period, were randomized to 12 weeks of double-blind treatment with BI 10773 5, 10, 25 mg qd, PBO, or to open-label metformin (MET) 1000 mg bid or max. tolerated dose The primary endpoint was the change in HbA_{1c} from baseline after 12 weeks of treatment.

Results: After 12 weeks, BI 10773 showed a dose-dependent and statistically significant reduction in FPG and HbA_{1c}, compared with PBO (Table). Patients on BI 10773 25 mg qd showed a comparable reduction in FPG and HbA_{1c} to those on MET. There was a significant reduction in body weight (BW) by ~2 kg on BI 10773, 0.75 kg on PBO, and 1.32 on MET. Compared with PBO, mean BW reductions were statistically significant for all BI 10773groups ($p<0.001$), but not for MET. The number of adverse events (AEs) was comparable among treatment groups (32.9% in PBO group, 38.8% in MET group, 29.1% in BI 10773 groups). Most frequently reported AEs (>2%) in the BI 10773 groups (average of AEs incidence of all BI doses) included pollakiuria (3.3% *vs* 0% in PBO), thirst (3.3% *vs* 0% in PBO) and nasopharyngitis (2.0% *vs* 1.2% in PBO). Frequency of urinary tract infection was low with BI 10773 (1.2%) and comparable to PBO (1.2 %) and MET (1.3%). Incidence of genital infections was low; mycosis was reported in 0.8% and pruritus in 1.2% on BI 10773 but none on PBO or MET. Six patients experienced at least 1 serious AE, of which none was drug related. (1.2 %vs 0% vs. 3.8 % Bi10773, PBO and MET, respectively).

Conclusion: In patients with T2DM, once daily administration of BI 10773, demonstrated a dose-dependent clinically meaningful reduction in glycemic control, comparable to those of metformin. Furthermore BI 10773 was associated with a clinically meaningful reduction in body weight. and showed a favourable safety profile.

	PBO	BI 5mg qd	BI 10mg qd	BI 25mg qd	PBO	MET
HbA1c(%)	0.09	-0.43*	-0.48*	-0.63*	0.07	-0.75*
HbA1c vs PBO (%)	-----	-0.52*	-0.57*	-0.72*	-----	-0.82*
FPG (mg/dL)	0.8	-23.3*	-28.9*	-31.1*	1.0	-29.7*
BW (kg)	-.75	-1.81*	-2.33*	-2.03*	-.75	-1.32

*p<0.001 vs PBO

Supported by: Boehringer Ingelheim GmbH & Co. KG

PS 80 Type 2 diabetes mellitus: new therapies

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Increased secretion of GLP-1 mediated by a newly discovered ligand for GPR119

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Background and aims: GPR119 is a G protein-coupled receptor expressed in pancreatic beta cells and the L-cells in the gastrointestinal tract in humans. In the intestine, activation of GPR119 results in the release of glucagon-like peptide-1 (GLP-1), which has been shown to improve glucose homeostasis through several mechanisms: e.g. stimulation of glucose-induced insulin secretion, inhibition of glucagon secretion and reduction of food intake. In search for activators of the GPR119 receptor our group recently identified a naturally occurring ligand with high affinity for the receptor (EC50 <1 μM), designated *XX* (patent protection; name of compound will be official at the Annual Scientific Meeting of EASD 2010). We aimed to investigate the impact of *XX* on endogenous GLP-1 secretion in healthy subjects.
Materials and methods: Six healthy Caucasian male subjects (age: 23±2 years (mean±SEM); BMI: 23±1 kg/m^2, fasting plasma glucose: 5.2±0.1 mM, HbA_1c: 5.2±0.04%) were investigated in a randomised single-blinded cross-over study. The subjects were given four different solutions on four different days using *glycerol* as vehicle: A) *XX*+*glycerol* (50 ml), B) *glycerol,* C) *XX*+*glycerol* and glucose (10 g), and D) *glycerol* and 10 g glucose. To prevent degradation in the stomach the solutions were delivered to the proximal jejunum through a tube placed distally to the ligament of Treitz. Plasma GLP-1, insulin and glucose were measured for four hours following delivery.
Results: The solution of *XX* (day A) elicited a significant greater initial GLP-1 response compared to *glycerol* (day B) ($AUC_{0\text{-}30\ min}$: 98±33 *vs.* -39±21 pM×30 min, p=0.036). We observed a significant greater initial plasma insulin response following delivery of *XX* (day A) compared to *glycerol* (day B) ($AUC_{0\text{-}30\ min}$: 809±168 *vs.* 321±104 pM×30 min, p=0.037). When the two solutions were administered along with glucose (day C and D) trends toward increased initial GLP-1 and insulin responses were observed.
Conclusion: These data show that *XX* induces secretion of GLP-1. This ligand might be a promising new way of stimulating endogenous GLP-1 secretion using an oral regime. Further studies defining doses, formulation and long-term effects are needed.

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Insulin sensitizer, BGP-15 prevents saturated fatty acid induced mitochondrial dysfunction

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Background and aims: Mitochondrial rotenone-sensitive NADH:ubiquinone oxidoreductase (complex I) activity and substrate switching were found to be diminished in obese and type 2 diabetic patients. Saturated long chain fatty acids, like palmitate inhibit complex I activity and result in increased mitochondrial ROS production. BGP-15 (R,S-O-(3-piperidino-2-hydroxy-1-propyl)-nicotinic-acid-amidoxime) is a new type insulin sensitizer drug candidate in human phase 2 developmental stage. It has been demonstrated that BGP-15 facilitates the expression of inducible heat shock protein resulting in decreased activation of JNK, which is a common mediator of obesity associated insulin resistance. Affinity binding assay revealed binding of BGP-15 to NDUFS1, a 75 kDa iron-sulfure protein subunit of mitochondrial complex I. Now we report the effect of BGP-15 treatment on the activity of complex I and II and ROS production in palmitate and hyperglycemia exposed cells. We also aimed to characterize the possible docking site of BGP-15 at NDUFS1.
Materials and methods: The effects of BGP-15 on the activity of complex I and II was evaluated in human HaCaT keratinocytes and L6 murine myocytes using a specific electron acceptor, DCIP and NADH or succinate electron donor. ROS production was measured by MitoSox or Amplex Red fluorescent probes. Vina and Surflex programs were used for molecular docking.
Results: BGP-15 treatment resulted in a rapid increase in the activity of complex I while the activity of complex II was inhibited in hyperglycemia exposed cells. The elevated activity of complex I was maintained in the presence of the drug for days in hyperglycemic culture conditions. Palmitate treatment decreased the activity of complex I and increased the activity of complex II, and elevated mitochondrial ROS production in L6 myocytes. BGP-15 treatment restored the activity of complex I and reduced the activity of complex II. In addition super-oxide production was lowered back to the level of untreated control. Molecular docking analysis on the bacterial (Thermus Thermophylus) homologue of human NDUFS1 identified a specific binding site (DG = - 7.2 kcal/mol) for BGP-15.
Conclusion: Data indicate that BGP-15 ameliorates the effects of palmitate on complex I resulting in balanced substrate oxidation and reduced ROS production. Molecular docking results support the concept that BGP-15 may target complex I, the main substrate entry and ROS producing site of mitochondria. Prevention of saturated fatty acid induced mitochondrial dysfunction can significantly contribute to insulin sensitizing effect of BGP-15.
Supported by: GOP 1.1.1

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GFT505, a new PPARα/δ agonist improves lipid and glucose homeostasis in prediabetic patients with atherogenic dyslipidaemia and/or impaired fasting glucose

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GFT505 is a drug candidate for the treatment of metabolic disorders associated with metabolic syndrome and Type 2 diabetes. GFT505 and GFT1007, its main active circulating metabolite, activate the PPARα and PPARδ subtypes. GFT505 potently reduces plasma triglycerides and total cholesterol and increases HDL cholesterol in animal models of dyslipidemia. GFT505 prevents the development of atherosclerotic plaques and has insulin-sensitizing properties in mice. In Phase I trials in healthy volunteers, GFT505 was well tolerated with no clinically relevant emergent adverse event. The maximum tolerated dose in humans has not been reached while a reduction in plasma triglycerides and increase in HDL-C was observed at doses from 40 to 100 mg/d. The safety of GFT505 was confirmed in the ensuing phase IIa trials. In a double blind, placebo controlled phase IIa trial conducted in 98 patients with atherogenic dyslipidemia (high triglycerides, low HDL-C), treatment with GFT505 at 80 mg/day for 28 days led to a 21 % (p=0.0027 vs placebo) reduction in triglycerides and a 9 % (p=0.003 vs placebo) increase in HDL-C. GFT505 significantly reduced ApoCIII (-17 %, p=0.04) and ApoB (-7 %, p=0.02), increased ApoA1 (+6%, p=0.002) and ApoA2 (+15 %, p<0.0001) and reduced fibrinogen and haptoglobin. These effects are comparable to those reported with fibrates, such as fenofibrate, in this population. In contrast, GFT505 did not induce clinically significant effects on homocysteine and creatinine. Finally, GFT505 reduced two distinct indicators of liver dysfunction: alanine aministransferase (ALAT, -15 % vs placebo, p=0.02) and Gamma Glutamyl Transpeptidase (-20 % vs placebo) while it did not affect plasma levels of aspartate aminotransferase. In a phase II clinical trial conducted in 47 patients with impaired fasting plasma glucose, impaired glucose tolerance and abdominal obesity, treatment with GFT505 (80mg/day) for 28-days led to a significant reduction in fasting plasma glucose levels (-5% vs placebo, p=0,03). In parallel, significant reductions in fasting plasma insulin (-25% vs placebo, p=0,009) and C-peptide (-11% vs placebo, p=0,03) were also obtained. Thus, the HOMA insulin-resistance index (HOMA-IR) was significantly reduced by -31% vs placebo (p=0,0027). Moreover, a beneficial effect of the compound on plasma lipids was observed with a significant reduction of LDL-C (-11% vs placebo, p=0,0049) and triglycerides (-25% vs placebo, p=0,0003) and an increase in HDL-C (+9% vs placebo, p=0,003). Finally, treatment with GFT505 led to a significant reduction in markers of inflammation (fibrinogen, -10%, p=0,0128; haptoglobin, -16%, p=0,007) while homocysteine levels remained unchanged. These first clinical trials position GFT505 as a new efficient and safe drug candidate for the treatment of prediabetic patients with impaired fasting glucose and atherogenic dyslipidemia.

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P1738, a novel insulin sensitizer improves metabolic control with a favourable weight profile in mice

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Background and aims: Type 2 Diabetes is characterized by peripheral insulin resistance and insulin deficiency. While most of the current treatments have focused on improving glycemic control, measures that can prevent co-morbidities such as dyslipidemia, hypertension and obesity are useful in reducing cardiovascular disease. Thiazolidinedione (TZD) drugs, including rosiglitazone exhibit potent antidiabetic and insulin sensitizing effects. However, TZD therapy has been associated with adverse cardiovascular outcomes and weight gain. Therefore, insulin sensitizers with improved safety profile are urgently needed. In this regard, we have used a phenotypic screening paradigm, capable of selecting modulators of insulin sensitivity, and identified P1738 as the lead compound. The present study describes the *in vitro* and *in vivo* pharmacology of P1738.
Materials and methods: We screened our chemical library in the phenotypic assay of adipogenesis using 3T3-L1 cells. The positives were evaluated for glucose uptake in the insulin resistant adipocytes and were identified as hits. The antidiabetic efficacy of these hits was investigated in *ob/ob* and *db/db* mice. Further, cardiac and developmental toxicity was determined using the zebra fish model.
Results: P1738 (20 μg/ml) enhanced insulin-induced adipogenesis of 3T3 fibroblasts. P1738 stimulated glucose uptake (EC50 - 0.5 μM) in the insulin resistant adipocytes. P1738 did not cause activation of $PPAR_{\alpha/\gamma}$ receptors in transactivation assays. Chronic oral treatment of *ob/ob* mice with P1738 induced a dose-related reduction in plasma glucose (23% at 200 mg/kg) and triglyceride levels (18% at 100 mg/kg and above). An oral glucose tolerance test carried out on day18 revealed that P1738 improved ($p<0.01$) glucose tolerance similar to rosiglitazone. Interestingly, P1738 treatment did not induce weight gain in mice, at all the tested doses (50 to 200 mg/kg) compared to rosiglitazone treatment (5 mg/kg) which induced 12% gain ($p<0.001$). Further, P1738 treatment did not significantly affect hematocrit and plasma protein levels, in contrast to rosiglitazone which induced significant reduction ($p<0.001$). P1738 exhibited an excellent liver safety profile with no changes in weight or triglyceride levels, even at 200 mg/kg while rosiglitazone (5 mg/kg) caused hepatomegaly and 30% ($p<0.01$) increase in liver triglyceride levels. P1738 was also efficacious in *db/db* mice wherein administration of 100 mg/kg resulted in 26% reduction in glucose levels while rosiglitazone (5 mg/kg) induced 35% reduction. Importantly, while rosiglitazone caused significant ($p<0.05$) weight gain, animals exposed to P1738 did not register significant change in body weight. When administered to diet-induced obese mice, P1738 did not cause weight gain while rosiglitazone induced significant increase during the same period. In a zebrafish model, P1738 (100 μg/ml) did not induce any adverse effects whereas pericardial edema was observed with rosiglitazone (25 μg/ml).
Conclusion: This study shows that P1738 is a novel insulin sensitizer with no $PPAR_{\alpha/\gamma}$ transactivation potential. Improvement in glycemic and extraglycemic parameters caused by P1738, in mouse models of obesity, is associated with a favorable weight profile. Thus P1738, with its unique pharmacology and improved safety profile, may represent an alternative treatment for Type 2 Diabetes.

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A selective GPR40 agonist, TAK-875, stimulates glucose-dependent insulin secretion without beta cell toxicity and decreases blood glycosylated haemoglobin levels in diabetic rats

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Background and aims: GPR40 is highly and dominantly expressed in pancreatic beta cells and is activated by medium- to long-chain free fatty acids (FFAs) to potentiate glucose stimulated insulin secretion. While GPR40 is an attractive target for Type 2 diabetes, it is unclear whether agonists or antagonists represent the best therapeutic approach. TAK-875 is a novel, oral, and selective small molecule agonist of GPR40 under development as a once-daily treatment for type 2 diabetes. In this study, pharmacological effects of TAK-875 were examined.
Materials and methods: EC_{50} for inositol monophosphate (IP) production was assessed in Chinese hamster ovary cells expressing human GPR40 (hGPR40-CHO). Acute effects (2h) of TAK-875 and glibenclamide were measured in rat insulinoma INS-1 833/15 cells. Chronic effects (72h) of TAK-875 and FFAs were measured in rat insulinoma INS-1 832/13 cells. Oral glucose tolerance test (OGTT) was performed in male N-STZ-1.5 diabetic rats (18w). Measurement of metabolic parameters after chronic treatment with TAK-875 for 6 weeks were performed in female Wistar fatty (WF) rats (14w).
Results: TAK-875 (EC_{50}=0.072 μM) showed over 400-fold stronger agonist activity compared to oleic acid (EC_{50}=29.9 μM) in IP production assay in hGPR40-CHO cells. In rat insulinoma INS-1 833/15 cells, TAK-875 increased intracellular IP and calcium concentration, indicating that the compound activates Gαq signaling pathway. Unlike the sulfonylurea glibenclamide (10 μM), the insulinotropic action by TAK-875 (10 μM) in INS-1 833/15 was glucose-dependent. Chronic exposure to TAK-875 (100 μM) for 72h in INS-1 832/13 cells did not affect insulin secretion, insulin content, or caspase 3/7 activity, while similar exposure to palmitic acid (1 mM) decreased both insulin secretion and insulin content, and increased caspase 3/7 activity. In an oral glucose tolerance test in N-STZ-1.5 diabetic rats, TAK-875 showed dose dependent improvement of glucose tolerance and potently augmented insulin secretion. Chronic treatment with TAK-875 in WF rats dose dependently decreased glycosylaed hemoglobin with no effects on body weight or pancreatic insulin content.
Conclusion: Our results support the concept that TAK-875 therapy may be an effective treatment of Type 2 diabetes without causing beta-cell toxicity.

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Chemokine receptor 2 antagonist CCX140-B in Phase 2 for type 2 diabetes

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Background and aims: CCX140-B is a highly specific oral chemokine receptor 2 (CCR2) antagonist in Phase 2 development for treatment of type 2 diabetes mellitus. Increased adiposity leads to elevated recruitment of inflammatory monocytes/macrophages into adipose tissue. Concurrent with increased adipose macrophage numbers, local and systemic elevations in inflammatory markers, such as TNFα, IL-6, and monocyte chemoattractant protein-1 (MCP-1)/chemokine ligand 2 (CCL2) are also seen and these inflammatory mediators have been shown to impair insulin sensitivity in multiple tissues. Studies in rodent models of insulin resistance and type 2 diabetes have indicated that the CCR2:MCP-1 axis is a primary control point for the entry of inflammatory macrophages in the adipose tissue of obese subjects.
Materials and methods: Male C57Bl/6 mice were placed on high-fat diet at 6 weeks of age for 18 to 24 weeks. Metabolic profiling (blood glucose, insulin, adiponectin) was determined in fasted mice. CCR2 antagonist or placebo was delivered once daily via subcutaneous injection for 7 to 28 days. Adipose tissue macrophage numbers were determined by fractionation of epididymal fat pads followed by flow cytometry to identify macrophages. Preclinical studies were followed by single and multiple ascending dose placebo-controlled Phase 1 clinical trials of the CCR2 antagonist CCX140-B at doses ranging from 0.05 mg to 10 mg in 88 healthy volunteers.
Results: Daily treatment with CCR2 antagonist significantly improved fasting glucose and HOMA-IR levels in obese, diabetic mice over 28 days of treatment (see table). Circulating adiponectin levels were significantly reduced in obese, diabetic mice and this was reversed by CCR2 antagonist treatment. The metabolic improvements correlated with a significant reduction in adipose tissue macrophage numbers. CCL2 levels were unchanged in mice treated with CCR2 antagonist. Additionally, circulating populations of monocytes were unchanged in the mice. CCX140-B was well tolerated in Phase 1 clinical trials. No serious adverse events (AEs) or withdrawals due to AEs were observed. No clinically significant laboratory abnormalities, vital signs, or ECG changes were observed. CCX140 pharmacokinetics were relatively linear across the dose range, with T_{max} of 1.4 to 3.2 hr and plasma half life of 40 to 58 hrs. Plasma MCP-1 levels, as well as circulating monocyte populations were unchanged after CCX140-B dosing, in contrast to results observed with other CCR2 antagonists. Based on the encouraging results from preclinical and Phase 1 clinical trials, a Phase 2 study in subjects with type 2 diabetes mellitus was initiated.
Conclusion: Oral CCR2 antagonist treatment improved metabolic function in mice without a corresponding increase in MCP-1. CCX140-B was well tol-

erated in Phase 1 with an attractive PK profile suitable for 5 to 10 mg once daily oral treatment.

CCR2 Antagonist Effect on Metabolic Profile in C57Bl/6 Mice (mean ± SD)

Variable	Normal Lean Mice	Vehicle-Treated Obese Mice	CCR2 Antagonist Treated Obese Mice
Fasting Blood Glucose (mg/dL)	105 ± 5	205 ± 9	140 ± 9*
Serum Adiponectin (ng/mL)	18.0 ±1.1	8.0 ± 0.5	14.3 ± 0.5*
HOMA-IR	1.7 ± 0.5	39.5 ± 9.0	9.4 ± 1.7*
Adipose Macrophages (cells/g)	34000 ± 9300	65000 ± 8500	27000 ± 6500*

* $p < 0.005$ vs vehicle

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Imeglimin, a novel glimin oral antidiabetic, exhibits good glycaemic control in type 2 diabetes mellitus patients

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Background and aims: Imeglimin is the first in the new glimin class of oral antidiabetic agents that targets insulin resistant organs and addresses beta cell failure. In decreasing mitochondrial oxidation, imeglimin inhibits excessive hepatic glucose production and restores peripheral glucose uptake as well as glucose-dependent insulin secretion. We investigated imeglimin's effects on glycaemic control compared with metformin in T2D patients.
Material and methods: Two phase II studies were conducted. In one, a 4-week repeat dose of imeglimin (2000 mg once daily [od], N=20; 1000 mg twice daily [bid] N=19) was compared with metformin (850 mg bid, N=19) over 4 weeks on glucose AUC during an OGTT. In the other, two daily doses of imeglimin (500 mg bid, N=31; 1500 mg bid, N=31) were compared with placebo (N=33) and metformin (850 mg bid, N=33) over 8 weeks and AUC glucose during a prolonged meal test, fasting plasma glucose (FPG) and HbA_{1c} were recorded.
Results: Baseline-adjusted changes in the OGTT AUC were -33% for imeglimin bid ($p<0.0001$), -30% for metformin ($p<0.0004$) and -10% for imeglimin od ($p=0.0305$). In the second study, the least square mean changes in $AUC_{0\text{-}6h}$ were significantly different from placebo for imeglimin 1500 mg and metformin with no statistical difference between the two treatment groups. Decreases in FPG and HbA_{1c} were observed for imeglimin 1500 mg and metformin. Only limited efficacy of the imeglimin 500 mg dose was noted. A greater response in all glycaemic parameters was generally observed for treatment naïve subjects compared with those previously treated and those with more severe hyperglycemia (HbA_{1c} ≥8%) compared with less severe hyperglycemia (HbA_{1c} <8%).

	Mean Change from Baseline (Standard Error of the Mean)							
Parameter	**Imeglimin 500 mg bid**		**Imeglimin 1500 mg bid**		**Metformin 850 mg bid**		**Placebo**	
$AUC_{0\text{-}6h}$ glucose (mmol/L)								
Overall	103.4	(158.5)	-365.7	(179.5)	-629.4	(144.7)	463.1	(165.1)
p-value	0.086		0.003		<0.0001		--	
Treatment naïve	-58.1	(153.8)	-515.9	(349.5)	-672.3	(191.1)	855.7	(159.1)
FPG (mmol/L)								
Overall	0.24	(0.35)	-0.88	(0.29)	-1.39	(0.21)	0.75	(0.35)
Treatment naïve	-0.15	(0.07)	-1.13	(0.29)	-1.63	(0.19)	1.21	(0.22)
HbA_{1c} (%)								
Overall	0.37	(0.18)	-0.13	(0.11)	-0.26	(0.09)	0.29	(0.12)
Treatment naïve	-0.40	(0.08)	-0.54	(0.07)	-0.44	(0.14)	0.11	(0.1)

No serious or severe adverse events associated with imeglimin were reported. No imeglimin-related adverse events were noted at its highest dose. In addition, no clinically significant changes in laboratory parameters, vital signs or ECGs were observed.
Conclusions: It was demonstrated that imeglimin was as effective as metformin at reducing $AUC_{0\text{-}6h}$, FPG and $HbA_{1c,}$ with no safety issues. Imeglimin is suitable for use as monotherapy at diagnosis of T2D and may be effective at any stage in the T2D continuum, from diagnosis through to disease complications. Because of its unique mechanism of action it may be well suited for combination therapy with most other classes of antidiabetic agents.

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A multicenter, randomised, placebo-controlled, paralled group, double-blind, Phase II trial to evaluate the efficacy and safety and to determine the optimal dose of LC15-0444 in Korean subjects with type 2 diabetes

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Background and aims: The objective of this study was to evaluate the efficacy and the safety of a novel dipeptidyl peptidase-4 (DPP-4) inhibitor, LC15-0444 and determine the optimal dose in Korean subjects with exercise/diet-controlled type 2 diabetes mellitus.
Materials and methods: In a double-blind, randomized, multicenter, parallel group, dose-range finding study, 145 patients (91 men and 54 women) with median age 53 years and with median BMI 25.1 kg/m^2 participated the study. Median fasting plasma glucose at baseline was 145 mg/dL and median HbA1c was 7.9%. Median duration since diagnosis of diabetes was 3 years. After 2 weeks of exercise/diet program and 2 weeks of placebo period after that, subjects were randomized to one of 4 groups, placebo, 50, 100 and 200 mg, for 12-week active treatment period.
Results: All three doses of LC15-0444 reduced HbA1C level from baseline to week 12 significantly compared to placebo (-0.06 vs. -0.98, -0.74, -0.78% in placebo, 50,100 and 200 mg respectively), with no significant differences among different dosings. Subjects with higher HbA1c level(≥8.5 %) at baseline experienced greater HbA1c reductions compared with the subjects with lower HbA1c level(<8.5%). Insulin secretory function assessed by HOMA-β, and C-peptide, and insulinogenic index was significantly improved by LC15-0444 treatment, and insulin sensitivity, assessed by HOMA-IR, was also significantly improved after 12 weeks of treatment. For lipid parameters, 50 and 200 mg groups showed significantly reduced total cholesterol and LDL-C level at week 12 from baseline compared with placebo. All 3 dosing groups did not have any effects on body weight nor on waist circumference, and the incidence of adverse events was similar in all four groups.
Conclusion: Once-daily LC15-0444 monotherapy for 12 weeks improved glycemic control in HbA1c, FPG, post oral glucose tolerance test (OGTT) glucose, improved measures of β-cell function and insulin sensitivity, and was well tolerated in Korean subjects with type 2 diabetes.
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The new dual glucagon-GLP-1 agonist ZP2929 improves glycaemic control and reduces body weight in murine models of obesity and insulin resistance

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Background and aims: Oxyntomodulin is released from L cells of the small intestine in response to meal ingestion, and is believed to exert its biological effects by activating both the GLP-1 receptor and the glucagon receptor, i.e. by acting as a dual glucagon-GLP-1 agonist. ZP2929 is a potent dual glucagon-GLP-1 receptor agonist with a pharmacokinetic profile compatible with once daily dosing. The effect of ZP2929 was studied in eight months high fat fed C57Bl/6J mice, as an animal model of insulin resistance and in four weeks high fat fed C57Bl/6J mice, as a model of obesity. ZP2929 was compared to vehicle and exendin-4.
Materials and methods: To assess the effect of ZP2929 on insulin resistance, eight months high fat fed C57Bl/6J male mice were randomized into groups with similar average fasting glucose, and treated twice daily s.c. with ZP2929 (10 nmol/kg), exendin-4 (10 nmol/kg), or vehicle. After 3 weeks of treatment,

following an initial blood sample for the determination of fasting blood glucose level, oral glucose tolerance tests were performed. To investigate the effect of ZP2929 on body weight gain, four weeks high fat fed C57Bl/6J male mice were randomized into groups with similar average body weight and treated twice daily s.c. with ZP2929 (0.6, 1.6, 3.2, 6.4 and 12.7 nmol/kg), exendin-4 (0.2, 0.5, 2, 5, 10 and 20 nmol/kg) or vehicle. Before treatment and after four and six weeks of treatment, blood samples were taken and blood lipids (low and high density lipoproteins (LDL and HDL), total cholesterol (TC) and triglycerides) measured.
Results: In eight months high fat fed C57Bl/6J mice, treatment with ZP2929 and exendin-4 for three weeks significantly ($p<0.05$) prevented the increase in fasting blood glucose seen in vehicle-treated animals. ZP2929 and exendin-4 improved glucose tolerance (measured as decrease in the area under the glucose curve) by 15.7 % and 30.3 %, respectively. In four weeks high fat fed C57Bl/6J mice, treatment with ZP2929 and exendin-4 significantly ($p<0.05$) and dose-dependently reduced body weight gain over time, compared to vehicle-treated animals. ZP2929 (12.7 nmol/kg) was significantly ($p<0.05$) more efficient than a similar dose of exendin-4 (10 nmol/kg) in preventing body weight gain. Plasma lipid parameters demonstrated a tendency to increase over time in vehicle-treated animals. At the two highest doses, ZP2929 and exendin-4 reduced the increase in plasma LDL, HDL and TC.
Conclusion: In conclusion, the new glucagon-GLP-1 agonist ZP2929 improves glucose control, markedly decreases body weight gain and improves blood lipid profile in murine models of insulin resistance and obesity.

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Long-term effect of amylin on the glucose metabolism of extrapancreatic tissues in insulin-resistant and type 2 diabetic states

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Background and aims: Amylin is glucose dependently co-secreted with insulin from the pancreatic β-cell, one another perhaps in an independent manner; it inhibits gastric emptying and glucagon release, reduces postprandial hepatic glucose productions, and shows anorexic effects. In type 2 diabetes, amylin secretion could is impaired. We studied the effect of amylin treatment on parameters related to the glucose metabolism of extrapancreatic tissues, in insulin-resistant (IR) and type 2 diabetic (T2D) rat models, compared to normal (N) animals.
Materials and methods: Male Wistar rats were used. IR was induced by chronic feeding -8 weeks- with standard chow combined with D-fructose (20% in the drinking water). T2D was developed by streptozotocin injection (100 μg/g bw) at birth. IR, T2D and N were treated (3 days) with saline (control) or 100 pM amylin (n=5-10 rats/group), through an osmotic pump; in fed conditions, blood samples were taken before and by the end of treatment for plasma glucose and insulin (RIA) measurements; GLUT-2 and GLUT-4 expression -Western blot and RT-PCR- was respectively studied in liver, muscle and fat; liver glycogen content, *soleus* muscle glycogen synthase *a* (GS*a*) -UDP-[^{14}C]glucose incorporation into glycogen- and isolated adipocytes glucose transport (GT) -2-deoxi-D-[1,2-^{3}H]glucose uptake-, were measured.
Results: The liver of IR (n=7 rats) and T2D (n=5), both showed GLUT-2-mRNA lower than (0.23±0.03 and 0.16±0.1 times N-control; $p<0.001$) and protein (overall mean: 62±6% N-control, $p<0.001$); amylin further reduced ($p<0.01$) mRNA in IR, without altering that in T2D; while failing to modify GLUT-2-protein in either group, amylin increased it in N (182±15% N-control, $p<0.001$). Respect N, liver glycogen content in IR (193±20 μg/mg protein) and T2D (230±18 μg/mg protein) was close to half lower ($p<0.01$); amylin increased the value in IR and T2D (573±66 μg/mg protein and 398±4, $p<0.01$ *vs* respective control). Muscle Glut-4-mRNA (n=10 rats) in IR and T2D were higher and lower, respectively, than in N (both $p<0.01$); amylin failed to modify the level in N or T2D, but apparently increased it in IR (1.49±0.39 times IR-control); Glut-4-protein was higher in IR (243±39% N-control $p<0.05$) and slightly lower in T2D ($p<0.05$); amylin, further reduced it in T2D (72±4% T2D-control, $p<0.001$) while increased it in IR (157±15% IR-control, $p<0.05$). GS*a* in IR and T2D was lower (38±14% N and 40±13%, respectively; $p<0.05$), and amylin normalized it in IR (53±3% N). In adipose tissue (n=11 rats), GLUT-4-mRNA in IR and T2D was lower (both $p<0.02$ *vs* N), while the protein was higher in T2D ($p<0.001$ *vs* N); amylin reduced GLUT-4-protein in N, but increased it in IR and T2D ($p<0.01$). GT in IR (8.1±0.5 fmol/10^5 cells, n=6) was lower ($p<0.01$ *vs* N) and that in T2D (45.1±2.7, n=23) higher ($p<0.001$); amylin normalized it in both groups, by respectively increasing ($p<0.02$) and reducing ($p<0.001$) the value. No major changes in plasma parameters were detected in any group after treatment.
Conclusion: In insulin-resistant and type 2 diabetic states, amylin could exert a restoring action on the deleterious glucose metabolism of extrapancreatic tissues participating in the glucose homeostasis, by perhaps increasing insulin sensitivity through its normalizing effect upon their altered glucotransporters expression.
Supported by: FIS, CIBERDEM, ENDODIAB

PS 81 Therapeutic alternative approaches to type 2 diabetes mellitus

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Vitamin D and calcium supplementation in adults at risk for type 2 diabetes. The CADDM randomised controlled trial

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Background and aims: Vitamin D and calcium have been shown to modify risk of type 2 diabetes in observational studies, but evidence from trials is lacking. The aim of the study was to determine whether vitamin D or calcium supplementation either alone or in combination improve glucose homeostasis in adults with glucose intolerance.
Materials and methods: Ninety-two adults with fasting plasma glucose (FPG) ≥100 mg/dl or 2-hour glucose (2hPG) ≥140 mg/dl after 75 grams of dextrose and Hemoglobin A1c 5.8 - 7% were randomized in a 2x2 factorial design to 1 of 4 arms as follows: vitamin D3 (2,000 IU once daily) and calcium carbonate (400 mg twice daily), vitamin D3 and placebo-calcium, calcium and placebo-vitamin D or placebo-vitamin D and placebo-calcium between October 2007 and November 2009. Outcome was change in glycemia (hemoglobin A1c, FPG and 2hPG) from baseline to 16 weeks. [ClinicalTrials.gov Identifier: NCT00436475]
Results: Enrolled participants had a mean age of 57 years, BMI of 32 kg/m2, 25-hydroxyvitamin D of 32 ng/ml and A1c of 5.9%. The difference in 25-hydroxyvitamin D between vitamin D3 and placebo-vitamin D was 13.3 ng/ml (p<0.001). Participants assigned to vitamin D3 had a smaller rise in hemoglobin A1c compared to those on placebo-vitamin D (0.05 vs. 0.15% respectively; p=0.034). Fasting plasma glucose (-0.08 vs. 2.2 mg/dl; p=0.12) and 2hrPG (-10.1 vs. 0.54 mg/dl; p=0.14) also improved, but the difference between groups was not statistically significant. Combined vitamin D3 and calcium carbonate improved glycemia more than vitamin D3 alone or calcium alone compared to the group assigned to placebos (0.04 vs. 0.08 vs. 0.11 vs. 0.20% respectively).
Conclusion: In adults at risk for type 2 diabetes, supplementation with vitamin D3 attenuates the increase in glycemia that occurs over time. The addition of calcium carbonate appears to further improve glycemia. Our findings need to be confirmed in long-term randomized trials.
Supported by: National Institutes of Health

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The effect of vitamin D supplementation on beta cell function and insulin sensitivity during a mixed meal tolerance test in healthy humans

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Background and aims: Increasing evidence from animal and human studies suggests that vitamin D may play a role in modifying risks of diabetes and other autoimmune diseases. Potential mechanisms of action of vitamin D on glucose metabolism include direct effects on the beta cell function and insulin sensitivity. The aim of our study was to evaluate the effect of a short time, high dose 25-OH vitamin D [25(OH)D] supplementation on glucose metabolism in healthy humans.
Materials and methods: 35 healthy subjects (16 females / 19 males, 35±11.4 years, 24.1±3.8 kg/m² BMI) were randomised to vitamin D supplementation (140.000IU monthly) or placebo (almond oil). A standard liquid mixed meal tolerance test (MMTT) (6ml Isosource /KG bodyweight) was performed at baseline and after 3 months of treatment. Areas under the curve (AUC) for glucose, insulin and C-peptide from pre-meal to 120min after consumption were calculated as were the Matsuda index of insulin sensitivity.
Results: 25(OH) D serum levels increased from 27.2±11.0 (mean ±SD) to 60.2±21.1ng/ml in the treatment group (n= 17) whereas 25(OH) D levels decreased from 25.6±7.1 to 18.8±7.2 ng/ml in the placebo group (n=18) during the 12 week study period. In the group receiving 25(OH) D supplementation the AUC for glucose (12258.2±1760.6 vs. 11577.8±2024.1 mg/dl; p=0.020) and insulin (5656±3207 vs. 4935.5±3363 µU/ml; p=0.087) decreased, with the main improvement of the AUC delta glucose (43.5±23.0 vs. 25.6±15.8mg/dl; p=0.014) and AUC delta insulin (83.4±56.5 vs. 59.9±50.8µU/ml; p=0.003) from 0 to 30min of the MMTT. Changes in AUC for C-peptide did not reach significant levels. The Matsuda insulin sensitivity index values increased slightly from 11.1±5.3 to 12.6±9.5 (p n.s.) in the treatment group but decreased significantly in the placebo group from 15.1±13.3 to 10.4±5.9 (p=0.023). In both groups no clinically relevant adverse events occurred.
Conclusion: Our data show that vitamin D supplementation in apparently healthy humans with insufficient 25(OH)D levels improves beta cell function and insulin sensitivity over a short period of time and support the beneficial effects of vitamin D on glucose metabolism.
Supported by: an EASD/MSD grant

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Anti-diabetic effect of add-on gynostemma pentaphyllum tea therapy with sulfonylureas in randomly assigned type 2 diabetic patients

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Background and aims: In Vietnam, herbs have been used traditionally to treat diabetic patients. Our previous works on *Gynostemma pentaphyllum* (GP) have revealed the anti-diabetic effect of GP extract in normal rats, associated with a novel insulin releasing gypenoside - phanoside. In addition GP tea has been proven in our recent clinical study to possess anti-diabetic effect with good safety data in newly diagnosed T2D patients, and to have effect on insulin sensitivity. The aim of the study was to investigate the anti-diabetic effect of the traditional Vietnamese herb GP as add-on therapy with sulfonylurea (SU) in 25 drug-naïve type 2 diabetic (T2D) patients.
Materials and methods: After 4-week treatment with gliclazide modified-release preparation, 30 mg daily, all patients were randomly assigned into 2 groups to add-on GP tea or placebo tea, 6g daily, during eight weeks. Fasting plasma glucose (FPG), C-peptide and insulin levels, HbA_{1C} and oral glucose tolerance tests (OGTT) were measured before, during and after the treatment.
Results: After 4-week treatment with SU, FPG and HbA_{1C} significantly decreased (p<0.001), C-peptide and insulin levels increased, and lipid profile improved significantly. There were no statistically significant differences between the groups allotted to GP and placebo tea. The decrease in FPG after eight weeks was 2.9 ± 1.7 mmol/l in the GP group and 0.9 ± 0.6 mmol/l in the control group (p < 0.001). Therapy with GP tea also significantly decreased 30 and 120 minute OGTT post-load values. HbA_{1C} levels decreased approximately 2%-units in the GP group compared to 0.7%-unit in the controls (p=0.001). The glycometabolic improvements were achieved without any major change of circulating insulin and C-peptide levels. There were no changes in Homeostasis Model Assessment-Insulin Resistance, lipids, body measurements, blood pressure and no reported hypoglycemias, or acute adverse effects regarding kidney and liver parameters.
Conclusion: The results of this study show that GP tea in addition to SU could offer an advantage over the addition of other oral medication to treat type 2 diabetic patients.
Supported by: SIDA/SAREC,

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Silibinin reverses insulin resistance in an animal model of high-fructose diet by an inhibition of glucose-6-phosphatase activity

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Background and aims: High fructose diet causes insulin resistance (IR) and alterations in glucose metabolism in Wistar rats. The flavonoid silibinin (SB) has recently showed important properties to be used in the treatment of type

2 diabetes. Previous studies *in vitro* of our group have shown that SB inhibits hepatic gluconeogenesis in perifused rat hepatocytes by an inhibition of glucose-6-phosphatase enzyme. The aim of this study was to explore the capacity *in vivo* of silibinin to reverse the particular alterations to IR induced in high fructose-fed rats, as well as the underlying mechanisms.

Materials and methods: Male Wistar rats were divided into two batches. One batch received a standard diet, and the other received a fructose-enriched diet. After 4 weeks, each group of rats was divided into two groups. One group received SB (50 mg/kg/day, i.p.) for additional two weeks, while the other received SB vehicle. Hepatocytes were isolated from starved rats, perifused at 37ºC and titrated with increasing substrate concentrations of dihydroxyacetone (DHA). We measured glucose concentrations in cellular perifusate and dihydroxyacetone phosphate (DHAP), glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P) in the cellular fraction. The effect *in vivo* of SB on glucose-6-phosphatase activity was also investigated.

Results: In perifused hepatocytes, fructose diet increased gluconeogenesis ($J_{Glucose}$) as compared with control by 25% (DHA 2.4 mM, p<0.001). Administration of SB normalized this metabolic alteration in fructose-fed rats (DHA 2.4 mM, p<0.001). Also, fructose-enriched diet increased DHAP, G6P and F6P concentrations at each steady state, giving a double relationship between $J_{Glucose}$ and DHAP, F6P and G6P. SB normalized this effect. On the other hand, high fructose diet increased glucose-6-phosphatase activity as compared with control by 10% (G6P 20 mM, p<0.05). SB fully reversed this effect. Regarding the kinetic parameters of glucose-6-phosphatase enzyme, fructose diet increased the maximal velocity (V_{max}; p<0.05) and decreased Michaelis-Menten constant (K_m; p<0.01). Administration of SB only reversed V_{max} (p<0.05) compared to fructose fed rats.

Conclusion: SB reverses metabolic alterations proper to insulin resistance in Wistar rats by an inhibition of glucose-6-phosphatase enzyme. These results suggest that SB could be beneficial as complementary therapy for the treatment of type 2 diabetes.

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The natural flavonoid Resveratrol reduces hepatic gluconeogenesis and respiration by a direct and a non-gene modification manner

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Background and aims: Resveratrol is a polyphenolic flavonoid with potent antioxidant activity. It is present in a variety of plants, notably berry fruit, and has emerged as a promising chemotherapeutic molecule due to its health benefits, especially in cancer, type 2 diabetes, cardiovascular and neurological diseases. The diverse biological effects of resveratrol refer to its ability to target many intracellular molecules, mainly through the activation of sirtuins, a class of NAD^+-dependent deacetylases that affect multiple transcription factors and other protein targets.To investigate other possible mechanisms of action of Resveratrol without involvement of gene activation in the liver, the most important organ of insulin resistance in type 2 diabetes.

Material and methods: Hepatocytes were isolated from 24h starved rats according to the method of Berry and Friend. They were perifused at 37ºC with Krebs-bicarbonate-calcium satured with O_2/CO_2 at a flow rate of 5 ml/min. Saturating concentrations of dihydroxyacetone (DHA) (0; 0.15; 0.30; 0.60; 1.20; 2.40; 4.80 mM) were used as exogenous substrate, in the presence or absence of 10 μM Resveratrol, to get seven consecutive steady states. Glucose, pyruvate and lactate concentrations were measured in cell perifusate for each steady state. We also studied the direct effect of Resveratrol on oxygen consumption rate (JO_2) using mitochondria isolated by differential centrifugation from livers of fed Wistar rats. JO_2 was measured polarographically at 37° using a Clark-type oxygen electrode, with either glutamate/malate (GM) or succinate/ malate (SM) as energy substrates of complex I or II, respectively.

Results: In perifused hepatocytes, Resveratrol (10 μM) inhibited hepatic glucose output ($J_{glucose}$; p<0.01) by 28% and glycolysis (J_{L+P}), determined by the addition of lactate+pyruvate, by 22% (DHA 2.4 mM; p<0.005). According to these results, we obtained an inhibition of total DHA metabolic flux ($J_{2glucose+L+P}$) by 25% (p<0.005) and a decrease of cytosolic NADH/NAD^+ redox state, estimated as the lactate/pyruvate ratio, of nearly 25 % as well (p<0.005). Resveratrol did not significatively affect the state 4 of respiration whatever the respiratory substrates used. On the contrary, the flavonoid inhibited the phosphorylating state 3 (in presence of ADP) by 15% with either substrate.

Conclusion: Resveratrol, a potent antioxidant flavonoid, is recently investigated for its therapeutic and preventive potential in age-related diseases like type 2 diabetes. For the first time we demonstrate that this compound exerts direct effects upon hepatic metabolism, *i.e.* inhibitions of hepatic gluconeogenesis and cell respiration. This acute response could be beneficial for the treatment of such metabolic disorders.

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A prescribed Chinese medicine improves glucose profile and ameliorates oxidative stress in GK rats fed with high fat diet

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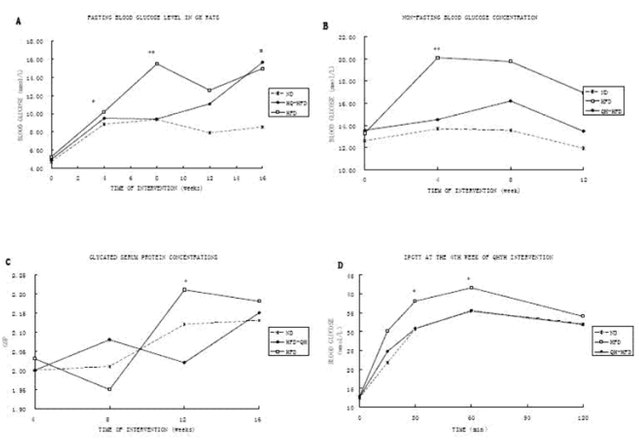
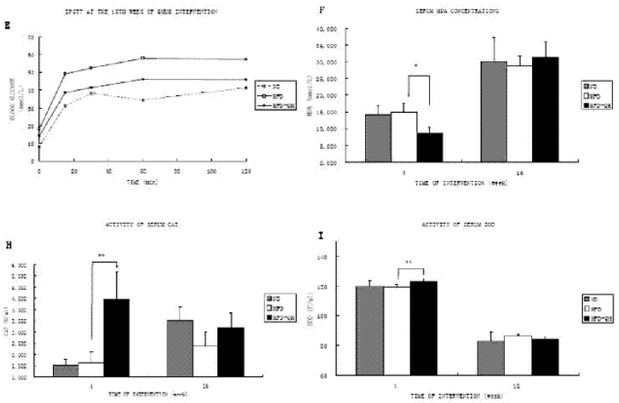
Background: The intrinsic anti-oxidative defence system of pancreatic β cells is fragile and oxidative stress is an underlying mechanism of β cell dysfunction. High-fat diet increases oxidative stress and exacerbates hyperglycemia in GK rat. We previously observed the prescribed traditional Chinese medicine preparation "Qing Huo Yi Hao" (QHYH) decreased urinary micro-albumin excretion in type 2 diabetic patients and also demonstrated its anti- oxidative activity by electron paramagnetic resonance. Recently we confirmed that QHYH protect endothelial cells from high glucose induced ROS production. So we hypothetized that QHYH might also protect β cell and improve glucose profile. We use GK rats fed with high-fat diet treated by QHYH to determine whether QHYH will improve glucose metabolism and ameliorate oxidative stress.

Materials and methods: 4-week-old male GK rats were given high fat diet (4.8kcal/g, 52% of the energy from fat) and divided randomly into 2 groups: QH group(n=10), rats were administered QHYH solution twice a day (3ml/Kg/d); and control (HFD) (n=10). Meanwhile, rats (ND) (n=10) fed normal chow (3.2kcal/g, 12% of the energy from fat)as normal control. Blood glucose and glycated serum protein(GSP) concentrations were measured every 4 week. IPGTT were done in 5 rats of each group at 4th and 16th week. Serum MDA concentration and activities of antioxidant enzymes were measured at the 4th and 16th week. Rats were sacrificed at the end of 16th week. Pancreas were taken out for morphological studies by immunohistochemistry to quantitatively determine β cell mass and relative volume. Nitrotyrosine and 8OHdG staining was also done.

Results: Of the QH group, fasting glucose level was significantly lower at 4th and 8th week(Fig 1A) , non-fasting blood glucose at the 4th week (1B) and GSP at the 12th week(1C) than HFD group. QHYH improved glucose tolerance by decreasing blood glucose level markedly at 15' and 30' after glucose load at the 4th week (1D). QHYH markedly decreased serum MDA concentrations(1F), increased serum CAT (1H) and SOD (1I) activities compared to HFD group only at the 4th week, however, GSH-Px activity(1G)was also markedly decreased. At the 16th week, neither glucose profile, nor glucose tolerance, nor serum MDA level or antioxidant enzyme activities was markedly different. Furthermore, in morphometry study, QHYH intervention did not restore the significant decrease of both beta cell mass and relative volume induced by high fat diet (ND vs HFD vs QH: 3.31±1.07mg vs 1.48±1.24mg vs 2.33±2.48mg, 27.6%±4.27% vs 19.4%±1.14% vs 18.1%±3.08%), nor with NT and 8OHdG staining.

Conclusions: QHYH improved glucose profile (4&8 weeks) and ameliorate oxidative stress (4 weeks) in GK rats fed with a high fat diet, which might be compromised by chronic glucotoxicity to pancreatic β cells





* P<0.05, **P<0.01 HFD-QH vs HFD (ANOVA-LSD)

# P<0.05, HFD-QH vs ND (ANOVA-LSD)

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Initial treatment with metformin + colesevelam provides greater glycaemic control than metformin alone in Hispanic patients with type 2 diabetes mellitus

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Background and aims: Colesevelam is indicated for glycemic control and LDL-C lowering in patients with type 2 diabetes mellitus (T2DM) and hypercholesterolemia, respectively. A 16-week (wk), randomized, double-blind, placebo-controlled, multinational study evaluated the effect of initial therapy with metformin + colesevelam in drug-naïve patients with T2DM.
Materials and methods: This post-hoc analysis evaluated the efficacy of metformin + colesevelam in a subpopulation of Hispanic patients (those self identified enrolled in Colombia, Mexico, and the US) included in the 16-wk study. Drug-naïve adults with T2DM (HbA_{1c} 6.5%-10.0%), LDL-C ≥2.6 mmol/L, and triglycerides (TG) <5.7 mmol/L were randomized 1:1 to metformin + colesevelam 3.75 g/d or metformin + placebo. Metformin was initiated at 850 mg/d and was uptitrated at Wk 2 to 1700 mg/d. Efficacy parameters included change in HbA_{1c} (primary) and LDL-C (secondary) from baseline to Wk 16. All efficacy analyses were performed with last observation carried forward at Wk 16.
Results: In total, 173 Hispanic patients were treated with metformin + colesevelam (n=85) or metformin + placebo (n=88). Mean baseline HbA_{1c} was 7.7% (metformin + colesevelam) and 7.6% (metformin + placebo). At Wk 16, the mean change from baseline in HbA_{1c} was significantly greater with metformin + colesevelam vs metformin + placebo (-1.2% vs -0.8%; treatment difference [TD]: -0.4%; *P*=0.001), resulting in significantly more patients achieving HbA_{1c} <7.0% (75% vs 56%; *P*=0.02). Metformin + colesevelam resulted in significantly greater mean reductions in LDL-C vs metformin + placebo (-22.8% vs -3.4%; TD: -19.4%; *P*<0.0001), resulting in significantly more patients achieving LDL-C <2.6 mmol/L (49% vs 14%; *P*<0.0001). There were significant LS mean changes from baseline with metformin + colesevelam vs metformin + placebo in non-HDL-C (-13.6% vs -3.3%; TD: -10.3%; *P*<0.0001), total cholesterol (-9.1% vs -1.2%; TD: -7.9%; *P*<0.0001), TG (median: 9.5% vs -11.1%; TD: 21.2%; *P*<0.0001), apoA-I (9.7% vs 5.4%; TD: 4.2%; *P*=0.01), and apoB (-11.4% vs -1.7%; TD: -9.8%; *P*<0.0001). Overall, metformin + colesevelam was well tolerated (Table).
Conclusion: Metformin + colesevelam may be an appropriate initial treatment option to improve glycemic and lipid control in Hispanic patients with T2DM.

Summary of Adverse Events

AEs occurring in ≥5% of patients, n (%)	Metformin + Colesevelam (n=89)	Metformin + Placebo (n=90)
Abdominal pain	5 (6)	7 (8)
Abdominal pain upper	5 (6)	2 (2)
Back pain	0	5 (6)
Constipation	6 (7)	2 (2)
Diarrhea	13 (15)	21 (23)
Dizziness	8 (9)	4 (4)
Dyspepsia	3 (3)	5 (6)
Headache	9 (10)	13 (14)
Hypertension	1 (1)	8 (9)
Influenza	11 (12)	9 (10)
Nausea	9 (10)	8 (9)

Supported by: Daiichi Sankyo, Inc.

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Plasma 25-hydroxyvitamin D concentration and metabolic syndrome in Chinese individuals - Shanghai Changfeng Study

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Background and aims: Several evidence suggests that 25-hydroxyvitamin D concentration is associated with metabolic syndrome (MetS), but the others consider that parathyroid hormone, but not vitamin D is related. We aimed to explore whether vitamin D or parathyroid hormone is associated with metabolic syndrome among a cross-sectional population of over 45 years old in Shanghai Changfeng community of China.
Materials and methods: The study population consisted of 538 inhabitants (40.5% men, median age 63.4 years) recruited from Changfeng community in Shanghai. A standard interview (included life style, diseases history through questionnaires). Anthropometrics (height, weight, waist and hip circumference, blood pressure), Laboratory parameters (including serum lipid, fasting plasma glucose [FPG], 2h postload plasma glucose [2hPG] after oral glucose tolerance test [OGTT], 25-hydroxyvitamin D and parathyroid hormone [Electrochemiluminescence assay, Roche E170], calcium and phosphorus) were conducted for each participant. Metabolic syndrome was defined using International Diabetes Federation criteria for Chinese.
Results: 495 inhabitants (40.4% men, median age 63.2 years) had completed data were included into analysis. The mean of plasma 25-hydroxyvitamin D concentration was 45.00±15.58nmol/l in this population. The frequency of metabolic syndrome is 33.7% (27.0% in men and 38.3% in women). Subjects with MetS had lower 25-hydroxyvitamin D concentration than those individuals without MetS (42.67nmol/l vs 46.18nmol/l, *P*=0.017). Compared with the highest tertile of 25-hydroxyviatamin D concentration(≥49.46nmol/l), the Odds ratio for metabolic syndrome in the middle (37.38-49.46nmol/l) and lowest tertile of 25-hydroxyviatamin D (≤37.38nmol/l) was 1.29 and 1.47 (95% CI 0.81-2.05 and 0.93-2.33) respectively. We observed that metabolic syndrome was negative associated with 25-hydroxyvatamin D (OR=0.963, 95%CI 0.945-0.981) in the logistic regression analysis. Significant inverse associations also existed between 25-hydroxyviatamin D concentration and metabolic syndrome (OR=0.941, 95%CI 0.901-0.983) when plus parathyroid hormone, blood calcium and phosphorus into model.
Conclusion: The occurrence of metabolic syndrome had increasing trend with the decline in the level of 25-hydroxyvitamin D. Lower 25-hydroxyvitamin D level is significantly associated with an increased risk of metabolic syndrome independent of parathyroid hormone in this Chinese population.
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PS 82 Conventional oral agents

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Effects of RSG/MET FDC on glycaemic control and BMD after 80 weeks of treatment in drug-naïve type 2 diabetes mellitus subjects

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Background and aims: Avandamet is a fixed-dose combination tablet comprised of rosiglitazone (RSG) and metformin (MET), which have complementary modes of action. Clinical studies have suggested that earlier use of combination therapy may improve long-term glycemic control. The purpose of this study (CT.gov NCT00386100) was to assess if RSG/MET significantly lowers A1c compared to MET alone, and if glycemic effects attained with AVM are durable over 80 weeks. A bone sub-study was added to this trial to further investigate the effects of RSG/MET on bone in light of an increased fracture rate in female subjects who received RSG in the ADOPT study. Presently, understanding of the clinical significance of these findings is incomplete, and the mechanism(s) for the observed increase in fractures are uncertain.
Materials and methods: In this double-blind, randomized study, RSG/MET was compared to MET in drug-naïve T2DM subjects with A1c ≥7.5% to ≤10.5%. Subjects (n=688) were randomized to either RSG/MET 4/500mg (max daily dose, 8/2000mg) or MET 500mg (max dose, 2000mg) and were assessed for 80 weeks. Doses were titrated at 4-week intervals up to Week 20 (unless mean daily glucose was <100mg/dL) and at 12-week intervals from Weeks 32 to 80 (unless A1c ≤6.5%). BMD by DXA at lumbar spine, total hip, femoral neck, trochanter, distal radius, and total body were assessed at Weeks 0, 20, 56 & 80.
Results: RSG/MET (-1.9%) was superior to MET (-1.4%) with respect to mean change from baseline A1c at Week 80 (-0.50%, p<0.0001). RSG/MET showed significant improvements in insulin sensitivity (HOMA-%S) at 80 weeks compared to MET alone (% treatment difference: 31.1%, p<0.0001). There were significantly greater reductions in fasting insulin, c-peptide, and free fatty acids for RSG/MET vs MET. No statistically significant between-group BMD reductions occurred in femoral neck, distal radius, or total body. Significant between-group BMD changes at Week 80 occurred at: lumbar spine in the overall, total female, and post-menopausal female groups; trochanter in the overall and total female groups; and total hip in all subgroups except post-menopausal females. MET alone was not associated with significant bone loss for the duration of the trial. Both agents were generally well-tolerated: withdrawals due to an AE occurred in 7% (25/344) and 5% (15/334) of RSG/MET and MET subjects. There were a total of nine on-therapy fractures (5 for RSG/MET, 4 for MET) reported as AE/SAEs. In the overall study, there were no unexpected adverse events.
Conclusion: RSG/MET significantly reduced A1c vs MET and maintained glycemic control over 80 weeks. These data confirm that RSG/MET is superior to MET in improving insulin sensitivity in drug-naïve subjects with T2DM. The observed BMD changes may be relevant to fracture risk in this population.
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Efficacy and safety of repaglinide and metformin combination therapy compared to repaglinide monotherapy in Chinese oral anti-diabetic drug (OAD) naïve type 2 diabetic patients

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Background and aims: The ADA and EASD recommend early initiation of combination therapy when HbA_{1c} > 8.5 %. Combination therapy with repaglinide and metformin is a good treatment option for type 2 diabetes. This trial was designed to investigate the efficacy and safety of this combination treatment compared to repaglinide monotherapy in Chinese OAD naïve type 2 diabetic patients with HbA_{1c} > 8.5 %.
Materials and methods: This was a 16-week, open-label, multicentre, randomised, active-controlled, parallel study involving 17 sites in China. Subjects were randomised 1:1 to receive either repaglinide in combination with metformin (1 mg/500 mg QD) or repaglinide alone (1mg TID), and then underwent a 6-week dose titration period followed by a 10-week maintenance period. During the dose titration period, subjects whose fasting plasma glucose (FPG) ≥ 6.1 mmol/L without clinically unacceptable hypoglycaemic episodes continued to titrate the daily dose until the FPG target of 4.4-6.1 mmol/L or the maximum recommended daily dose (repaglinide/metformin 4mg/500mg, TID; repaglinide 4 mg, TID) was reached.
Results: A total of 432 subjects (female 313, mean ± SD: age 49.9 ± 10 years, BMI 24.5 ± 3.0 kg/m^2, HbA_{1c} 10.8 ± 1.5 %) were exposed to trial drugs. After 16-weeks treatment, the glucose control was improved in both groups. Mean HbA_{1c} decreased from 10.9 ± 1.5 % at baseline to 6.4 ± 1.1 % at 16 weeks in the combination therapy group, and from 10.7 ± 1.5 % to 6.7 ± 1.0 % in the monotherapy group. The mean reduction was 4.5 ± 1.6 % point and 4.1 ± 1.6% point, respectively (P = 0.002). HbA_{1c} < 7 % was achieved in 78.9 % of the subjects with combination therapy, and in 69.6 % of those with repaglinide alone (P = 0.010). Compared to monotherapy, the combination treatment also achieved a superior outcome in FPG, 2-hour postprandial plasma glucose, mean 7-point plasma glucose, and mean prandial plasma glucose increment (Table). No major hypoglycaemia was reported during the trial, and the overall hypoglycaemia rate was 2.04 events per subject year in the combination treatment group and 1.35 events per subject year in the monotherapy group (P = 0.058). Adverse events reported during the trial were comparable between groups. At the end of the trial, mean weight gain was small in both groups; 0.2 ± 0.2 (mean ± SD) kg in the combination therapy group and 0.5 ± 0.2 kg in the monotherapy group, respectively (P = 0.322).
Conclusion: Combination therapy with repaglinide and metformin and repaglinide monotherapy improved glucose control significantly in OAD naïve Chinese type 2 diabetic patients with baseline HbA_{1c} > 8.5 %. However, combination treatment provided superior glycaemic control compared to repaglinide monotherapy. The safety profile was comparable between groups.

Comparision of glucose control parameters between groups

Glucose control parameters (mean ± SD), mmol/L		Baseline	After 16-week treatment	Change from baseline
Fasting plasma glucose	Repaglinide + Metformin	11.30 ± 3.20	6.34 ± 1.50**	-4.98 ± 3.23**
	Repaglinide	11.23 ± 3.56	6.71 ± 1.72**	-4.44 ± 3.50**
2-hour postprandial plasma glucose after breakfast	Repaglinide + Metformin	17.08 ± 5.34	9.18 ± 3.46	-7.86 ± 5.93
	Repaglinide	16.96 ± 5.24	9.61 ± 3.23	-7.30 ± 5.57
Mean 7-point plasma glucose	Repaglinide + Metformin	14.27 ± 3.93	7.39 ± 1.76**	-6.84 ± 3.95**
	Repaglinide	14.03 ± 3.86	8.16 ± 2.13**	-5.86 ± 3.82**
Mean prandial plasma glucose increment	Repaglinide + Metformin	4.13 ± 2.42	2.05 ± 1.85*	-1.99 ± 2.85*
	Repaglinide	3.87 ± 2.71	2.51 ± 1.88*	-1.35 ± 2.92*

*: P< 0.05; **: P< 0.01 between groups.

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Metformin therapy and outcomes of viral C cirrhosis in type 2 diabetic patients. A retrospective study

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Background and aims: Insulin resistance seems to play a crucial role in the poor prognosis associated with overweight and obesity for viral C cirrhosis. The aim of the study was to retrospectively examine whether metformin therapy (which activates AMP kinase and decreases insulin resistance) may be associated with a better prognosis in patients with type 2 diabetes.
Materials and methods: Between 1988 and 2007, 100 consecutive diabetic patients, 53 men, 61±11 years old, body mass index 27±5 kg/m^2, with ongoing viral C infection and cirrhosis were included in a screening program for

hepato-cellular carcinoma (HCC) detection including serum alpha foeto protein (AFP) measurement and ultrasound examination every 3 to 6 months. The patients were followed up for HCC and liver related death or hepatic transplantation.
Results: Diabetic patients were treated either by metformin (n=22) or not (insulin (n=28), insulin secretory drugs (n=20) or diet alone (n=30)). No statistically significant difference was observed at inclusion between the two groups. During a mean follow-up of 5.9±4.4 years, 38 patient developed an HCC and 38 had a liver related death. The 5-year HCC incidence was 10.9% and 29.8% in patients who received metformin or did not receive metformin respectively (log rank 7.47, p=0.006). In multivariate analysis, metformin treatment was associated with a reduced incidence of HCC (Odds ratio 0.23 [95% CI 0.06-0.96], p=0.04) whereas age (OR 2.33 [1.14-4.77], p=0.02) and serum AFP level (OR 1.02 [1.01-1.03], p=0.005) were predictive of HCC occurrence. Metformin therapy was also associated with a lower incidence of liver related deaths or transplantation (log rank 6.3, p<0.05).
Conclusion: In diabetic patients with cirrhosis and persistent HCV infection, metformin use is associated with a reduced risk of HCC occurrence and liver related death or transplantation.

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Treatment with pioglitazone plus insulin is well tolerated and shows metabolic benefits compared to a combination of insulin and metformin. Interim results from the PIOcomb study

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Background and aims: Addition of basal insulin to oral therapy in type 2 diabetes (basal insulin supported oral therapy, BOT) is a very common therapeutic strategy in Germany. Our investigation aimed at comparing a pioglitazone plus insulin (PI) regime with a metformin plus insulin (MI) treatment, and with triple therapy (PMI). Observation parameters included glycemic control, insulin sensitivity, insulin consumption, beta-cell preservation, and the intensity of chronic systemic inflammation.
Materials and methods: In an interim analysis of the double-blinded, multicentre PIOcomb study, we included 78 type 2 diabetic patients with previous insulin therapy (52 men, 26 women, age [mean±SD]: 63±8 years, BMI: 32.7±5.6 kg/m^2, diabetes duration: 9±5 years, HbA1c: 7.3±0.5%). Patients received an optimised and individualised regimen with insulin glargine (titration) and were randomised to an additional treatment with pioglitazone (2 x 15 mg/day) or metformin (2 x 850 mg/day), or a combination of both oral antidiabetic drugs. Efficacy parameters (HbA1c, insulin dosage, HOMA score, intact proinsulin, adiponectin, and hs-CRP) were assessed at baseline and after a six-month observation period.
Results: HbA1c remained stable in the dual therapy groups, and decreased in the PMI treatment arm (PI/MI/PMI: -0.1±0.6 %/-0.1±0.7 %/-0.5±0.6 %, p<0.05 vs. baseline). Improved insulin sensitivity was only demonstrated in the two groups receiving pioglitazone. The baseline and endpoint values of the observation parameters are provided in the Table. There were no differences in the reported number of hypoglycemic events between the groups (8/8/10).
Conclusion: Addition of metformin and/or pioglitazone to a basal insulin therapy with insulin glargine provided stable metabolic control in all cases, without increasing the risk for hypoglycemia. A significant improvement in insulin resistance and cardiometabolic syndrome, as indicated by related biomarkers and a reduction in daily insulin dose, was only observed with pioglitazone administration.

Observation parameters at baseline and endpoint (*: p<0.05 vs. baseline)

Parameter	MI	(n=25)	PI	(n=28)	PMI	(n=25)
	baseline	endpoint	baseline	endpoint	baseline	endpoint
HbA1c [%]	7.3±0.6	7.2±0.5	7.4±0.6	7.3±0.8	7.3±0.5	6.8±0.7*
Daily Insulin dose [IU]	37±17	41±21*	37±17	29±16*	36±21	28±22*
HOMA-IR	4.5±4.2	4.8±4.2	4.4±4.1	2.4±1.8*	3.3±2.9	1.8±1.4*
Adiponectin [mg/L]	4.2±2.4	4.1±2.2	4.0±2.5	12.6±8.6*	4.7±3.2	12.2±7.5*
Intact Proinsulin [pmol/L]	12±21	6±5*	7±5	5±3*	5±4	3±1*
hsCRP [mg/L]	3.6±2.7	3.3±2.7	3.3±2.7	2.5±1.9*	2.6±1.9	1.4±1.0*

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Comparison of the effects of pioglitazone vs. placebo when given in addition to standard insulin treatment in patients with type 2 diabetes mellitus requiring haemodialysis: interim results from the PIOren study

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Background and aims: Patients with type 2 diabetes mellitus and clinically significant kidney disease are usually treated with insulin. However, the modified pharmacokinetic insulin profile and vascular insulin resistance in patients with delayed renal insulin elimination frequently impairs a successful therapy and, additionally, results in increased oxidative stress and cardiovascular risk. Therefore, the aim of this study was to investigate the effect of the insulin sensitizer pioglitazone (PIO) vs. placebo treatment on total daily insulin requirements and the overall metabolic status in type 2 diabetes patients with renal failure requiring hemodialysis.
Materials and methods: The effect of pioglitazone (30 mg) vs. placebo was explored by an interim analysis of this multi-centre, randomized, double-blind study in 23 patients with Type 2 diabetes and kidney failure and dialysis therapy (16 male, 7 female, age (mean±STD): 68.2±8.8 yrs., HbA1c: 7.5±0.8 %, disease duration: 11.8±8.5 yrs.). Efficacy parameters collected before dialysis and after an overnight fast at baseline and after 6 months were: total daily insulin dose, HbA1c, fasting blood glucose, adiponectin, HDL, LDL, triglycerides, NT-proBNP and ultra filtrate volume.
Results: Application of PIO resulted in a significant decrease in insulin resistance as indicated by a reduction in daily insulin dose by 32 % (p<0.05 vs. baseline; placebo: -5 %., n.s.), and clinically relevant improvements in HbA1c (-0.62±0.75 %, p<0.01; placebo: +0.57±1.08 %, n.s.), fasting glucose (-56±73 mg/dl, p<0.05 vs. +20±35 mg/dl, n.s.), adiponectin (+6.9±8.4 mg/l, p<0.01 vs. +1.0±6.5 mg/l, n.s.), and triglycerides (-111±178 mg/dl, p<0.05 vs. +65±70 mg/dl, p<0.05). Slight improvements or no changes were seen with HDL, LDL, NTproBNP and the ultra filtrate volume. The absolute values at baseline and endpoint are provided in the Table.
Conclusion: Addition of pioglitazone to insulin in hemodialysis patients was well tolerated. Without changing the ultra filtrate volume, treatment with pioglitazone in addition to insulin in patients with late stage kidney failure requiring hemodialysis was associated with a lower insulin dose and with an improved glycaemic control and lower triglyceride levels, indicating a potential impact of pioglitazone on the long-term disease prognosis in late stage diabetes.

Observation parameters at baseline and endpoint (*: p<0.05 vs. baseline)

Parameter	Pioglitazone	(n = 14)	Placebo	(n = 9)
	Baseline	6 months	Baseline	6 months
Daily insulin dose [IU]	68.3±54.5	46.2±35.3*	53.3±18.5	50.5±17.5
HbA1c [%]	7.6±1.0	7.0±0.9*	7.4±0.6	7.9±0.6
Glucose [mg/dL]	178±61	122±32*	151±56	170±57
Adiponectin [mg/L]	10.0±4.9	16.9±7.3*	8.9±8.3	9.8±5.9
HDL [mg/dL]	32±10	36±10	35±10	32±9
LDL [mg/dL]	99±49	117±40	102±33	111±47
Triglycerides [mg/dL]	333±237	222±87*	244±113	309±140*
NT-proBNP [pg/mL]	6028±10144	4470±6294	7752±9427	14272±19287
Ultra filtrate volume [ml]	2402±920	2576±1021	2792±993	2923±1125

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PIOcomb study interim analysis: Pioglitazone added to insulin treatment reduces chronic systemic inflammation in patients with type 2 diabetes

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Background and aims: Combination therapy with insulin glargine and metformin is a common therapy in type 2 diabetes. The purpose of the PIOcomb study was to investigate, whether use of pioglitazone instead of metformin has additional impact on the underlying disease pathophysiology, and in particular on chronic systemic inflammation (CSI). CSI leads to macrovascular complications, the major cause of mortality in patients with type 2 diabetes.

Materials and methods: Here we report on an interim analysis of the double-blinded, multicentre PIOcomb trial (78 patients with previous insulin therapy, 52 men, 26 women, age [mean±SD]: 63±8 years, BMI: 32.7±5.6 kg/m^2, diabetes duration: 9±5 years, HbA1c: 7.3±0.5%). Patients were switched for 6 months to an individualised once daily insulin glargine injection (with forced titration to normal fasting glucose values) and were additionally randomised to receive either pioglitazone (PI; 2 x 15 mg/day), or metformin (MI; 2 x 850 mg/day), or a combination of both oral antidiabetic drugs (PMI). Efficacy parameters for this analysis (determined at baseline and after 24 weeks) were HbA1c, MMP9, hsCRP, E-selectin, fibrinogen, PAI-I, nitrotyrosine, and the NFkB mRNA expression of peripheral circulating monocyte/macrophages.
Results: Daily insulin dose increased with MI and decreased with PI and PMI, which was associated with stable or improved glycemic control (HbA1c, PI/MI/PMI: -0.1±0.6 %/-0.1±0.7 %/-0.5±0.6 %, PMI: p<0.05 vs. baseline). With PI and PMI, there were significant reductions in hsCRP and E-selectin and improvements were also seen for macrophage activation (NFkB) with PI and PAI-I with PMI. All other parameters showed tendencies for improvement with PI and PMI that did not reach the level of statistical significance. The baseline and endpoint values are provided in the Table. None of these beneficial changes were seen with MI treatment.
Conclusion: While reaching comparable glycemic control (compared to metformin) with lower insulin requirements, addition of pioglitazone to insulin glargine improved several biomarkers of chronic systemic inflammation and vascular function in patients with Type 2 diabetes. This may translate into a lower macrovascular mortality as has been previously indicated in the PROactive study.

Observation parameters at baseline and endpoint (*: p<0.05 vs. baseline)

Parameter	MI	(n = 25)	PI	(n = 28)	PMI	(n = 25)
	Baseline	Endpoint	Baseline	Endpoint	Baseline	Endpoint
HbA1c [%]	7.3±0.6	7.2±0.5	7.4±0.6	7.3±0.8	7.3±0.5	6.8±0.7*
hsCRP [mg/L]	3.6±2.7	3.3±2.7	3.3±2.8	2.5±1.9*	2.6±1.9	1.4±1.0*
MMP-9 [ng/mL]	615±365	673±415	522±171	516±222	577±266	534±179
E-selectin [ng/mL]	46±21	46±22	49±19	45±18*	46±16	41±15*
Fibrinogen [g/L]	3.7±0.8	3.6±1.0	3.4±0.9	3.3±1.2	3.6±1.2	3.4±1.0
PAI-I [ng/mL]	66±25	60±29	68±27	60±31	61±28	44±30*
Nitrotyrosine [nmol/L]	398±136	428±152	377±125	364±104	387±110	383±147
NFkB [RLU]	0.74±0.12	0.74±0.07	0.69±0.09	0.67±0.08*	0.69±0.09	0.67±0.09
Daily Insulin dose [IU]	37±17	41±21*	37±17	29±16*	36±21	28±22*

Supported by: Takeda Pharma

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Effect of acarbose on inflammatory parameters at baseline and after an oral fat load: a double-blind, placebo controlled trial
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Background and aims: To evaluate the effects of acarbose compared to placebo on inflammatory biomarkers in diabetic patients at baseline and after a standardized oral fat load (OFL).
Materials and methods: A multicenter, randomised, double-blind, controlled study was conducted; 188 type 2 diabetic patients were randomised to titrate acarbose til 100 mg three times a day or placebo. We evaluated at the baseline, and after 1, 2 and 7 months: body mass index (BMI), glycemic profile, fasting plasma insulin (FPI), post-prandial plasma insulin (PPI), homeostasis model assessment index (HOMA index), lipid profile, soluble intercellular adhesion molecule-1 (sICAM-1), interleukin-6 (IL-6), high-sensitivity C reactive protein (Hs-CRP), soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble E-selectin (sE-selectin). Furthermore, at the baseline and at the end of the study, all patients underwent OFL. Repeated measures analysis of variance (ANOVA), analysis of covariance (ANCOVA), and paired tests were used: a one-sample t test and two-sample t tests.
Results: We observed a significant decrease of body weight, and BMI in both groups (p<0.05, for both). An improvement of glycemic profile was observed in both groups, but acarbose gave a faster and better decrease of these parameters compared to placebo (p<0.01 for glycated hemoglobin, and p<0.05 for fasting and post-prandial glucose). No variation of FPI, or PPI were observed in control group, while there was a reduction of these parameters after 7 months with acarbose (p<0.05), even if no differences between the two treatments were recorded. Acarbose reduced HOMA index after 3, and 7 months (p<0.05, and p<0.01, respectively), while no modifications were registered in controls; moreover HOMA index obtained with acarbose was significantly lower than the value in control group (p<0.05). Neither of treatments influenced HDL-cholesterol; instead acarbose significantly reduced total cholesterol (p<0.05), and LDL-cholesterol (p<0.05) after 7 months compared to baseline, and with control group (p<0.05). No variation of tryglicerides (Tg) was reached in controls, while acarbose decreased Tg value after 3, and 7 months compared to baseline (p<0.05, and p<0.01, respectively), and with controls (p<0.05). We did not observe any variation of s-ICAM-1, sVCAM-1, IL-6, and Hs-CRP in controls, while there was a reduction of these parameters with acarbose (p<0.05 for all), even if no differences were obtained between the two groups. No variation of E-selectin was recorded in neither of groups. Regarding OFL, there was a decrease of blood glucose levels in both groups comparing OFL administered at baseline, and at the end of the study. There was an improvement of all lipid parameters in acarbose group, while there was only a reduction of Tg in control group. A reduction of sICAM, sVCAM, IL-6, Hs-CRP, and sE-Selectin was registered in both groups, even if acarbose had a longer effect in reducing inflammatory parameters during OFL.
Conclusion: Acarbose gave a faster and better improvement of glycemic, lipid profile and inflammatory parameters compared to placebo. Regarding OFL, acarbose had a longer effect in reducing inflammatory parameters compared to controls.

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Acarbose compared to placebo on insulin resistance biomarkers in a double-blind, placebo-controlled trial
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Background and aims: To evaluate the effects of acarbose compared to placebo on insulin resistance biomarkers in a randomised, double-blind, placebo controlled trial.
Materials and methods: We randomised 188 type 2 diabetic patients to titrate acarbose til 100 mg three times a day or placebo. We evaluated: body mass index (BMI), glycated hemoglobin (HbA_1c), fasting plasma glucose (FPG), post-prandial plasma glucose (PPG), fasting plasma insulin (FPI), post-prandial plasma insulin (PPI), homeostasis model assessment index (HOMA index), lipid profile, tumor necrosis factor-α (TNF-α), resistin (r), retinol binding protein-4, adiponectin (ADN), high-sensitivity C reactive protein (Hs-CRP) at the baseline, and after 1, 2 and 7 months. Furthermore, at the baseline and at the end of the study, all patients underwent euglycemic hyperinsulinemic clamp to evaluate M value and total glucose requirement (TGR); an oral fat load (OFL) was also performed at baseline, and after 7 months of therapy. Repeated measures analysis of variance (ANOVA), analysis of covariance (ANCOVA), and paired tests were used: a one-sample t test and two-sample t tests.
Results: We observed a significant decrease of body weight and BMI in both groups (p<0.05 for both). An improvement of $HbA_{1c,}$ FPG, and PPG was reached in both groups, even if acarbose gave a faster and better improvement of these parameters compared to placebo (p<0.01 for $HbA_{1c,}$ and p<0.05 for both FPG, and PPG). Placebo did not reduce FPI, or PPI, while acarbose decreased these parameters after 7 months of therapy (p<0.05), even if no differences between the two treatments were recorded. Acarbose significantly reduced HOMA index compared to baseline after 3, and 7 months (p<0.05, and p<0.01 respectively), and with controls after 7 months (p<0.05). Acarbose significantly decreased total cholesterol (p<0.05), tryglicerides (Tg) (p<0.01), and LDL-cholesterol (p<0.05) after 7 months compared to baseline, and with controls (p<0.05). We did not observe any variation of TNF-α in neither of groups. We obtained a reduction of r, and Hs-CRP after 7 months with acarbose (p<0.05, for both) but not with placebo, even if no differences were recorded in group to group comparison. Moreover, acarbose, but not placebo, gave a decrease of RBP-4, and an increase of ADN compared to baseline (p<0.05 for both), and the values obtained at 7 months were significantly better than the values obtained in control group (p<0.05). Regarding OFL, there was a decrease of blood glucose levels in both groups during OFL administered at the end of the study compared to OFL at baseline. There was an improvement of all lipid parameters in acarbose group, while there was only a decrease of Tg in control group. A reduction of RBP-4, TNF-α, and Hs-CRP,

and an increase of ADN were observed in both groups, even if acarbose had a longer effect in reducing inflammatory parameters during OFL. We reached an increase of M value, and TGR after 7 months ($p<0.05$ for both) with acarbose, but not with placebo.
Conclusion: We reached a faster and better improvement of glycemic and lipid profile with acarbose compared to placebo. Acarbose also improved insulin resistance biomarkers, while placebo did not decrease these parameters. Regarding OFL, acarbose had a longer effect in improving insulin resistance compared to control group.

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Efficacy of miglitol on postprandial glucose control assessed by continuous glucose monitoring

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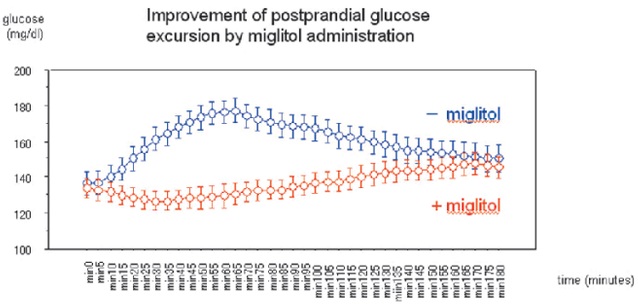
Background and aims: α-glucosidase inhibitors improve postprandial glucose control, but their precise efficacy has not been evaluated by continuous glucose monitoring (CGM). The present study was undertaken to reveal efficacy of miglitol on postprandial glucose control.
Materials and methods: Nineteen diabetic subjects (4 with type 1 and 15 with type 2; with ages of 58±11yrs, BMI 22.8±3.2kg/m^2, HbA1c 9.1±2.5%, glycated albumin 24.5±6.3%, and 1, 5-anhydroglucitol 6.5±3.9μg/ml, mean±SD, respectively) were studied with CGM on 2 consecutive days. By a cross-over manner, their usual therapies (10 with multiple insulin injections, 4 with oral hypoglycemic agents, and the remaining 5 with diet therapy alone) were rendered on one day, and miglitol 150mg/day was additionally administered on another day. These measurements were performed while the patients were admitted and given controlled diet comprising 50% of energy intake as carbohydrate.
Results: Averaged glucose levels on their usual therapies were 137±49 (pre-prandial), a peak of 177±49 observed at 60 min postprandial, and then the levels were gradually decreased to 151±57 mg/dl (at 180 min). The glucose levels on miglitol administration were 134±43 mg/dl (pre-prandial), and thereafter no apparent peak was observed until 180 min (P=0.0015 by two-way repeated-measures ANOVA, Figure). There were also significant differences in glucose levels from 20 to 120 min postprandial between these two situations. On their usual therapies, pre-prandial glucose levels at breakfast, lunch, and supper were 136±52, 136±48, and 137±52 mg/dl, and maximal postprandial levels were 181±49 (at 65), 160±51 (at 65), and 192±42 mg/dl (at 55), respectively. By miglitol administration, postprandial glucose levels displayed essentially similar pattern as shown in Figure, so that the efficacy of miglitol was most evident after dinner. Some of the measures of glycemic variability assessed by CONGA (Diabetes Technol Ther 7: 253, 2005) were improved by miglitol administration.
Conclusion: Postprandial glucose levels were significantly reduced from as early as 20 min and until 120 min by miglitol administration. The efficacy was most evident after dinner.



905

Gliclazide blocks cytotoxic effect of hydrogen peroxide

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Background and aims: Apoptosis, a programmed cell death, is needed to maintain homeostasis of the cell under physiological condition. However, this process can be induced under pathological conditions such as an increase in reactive oxygen species (ROS) production in the mitochondria. It is believed that ROS-induced apoptosis is responsible for loss of beta cell mass in diabetes. It was found that gliclazide - a member of sulfonylureas group- may protect pancreatic beta cells against apoptosis induced by oxidative stress. The aim of our study was to explore the antiapoptotic action of gliclazide *in vitro*.
Materials and methods: Evaluation of the impact of gliclazide on apoptosis induced by hydrogen peroxide of pancreatic and mammary gland tumor cells (PANC-1 and Hs578T) was assessed by the 1-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) colorimetric assay and the neutral red uptake (NRU) cytotoxicity assay. The effect of gliclazide on the level of ROS was measured by the 2',7'-dichlorodihydrofluorescein diacetate (DCFH2-DA) assay. N-acetylcysteine (NAC), that possesses antioxidant properties similarly to gliclazide, was used as a control.
Results: We observed that hydrogen peroxide induced a concentration- and time-dependent loss of viability of PANC-1 and Hs578T cells. Gliclazide significantly diminished cytotoxic effect of hydrogen peroxide on both cell lines. It was also found that either gliclazide and NAC inhibited generation of ROS in both cell lines. Interestingly, there were not significant differences in inhibition of ROS generation between gliclazide and NAC after 24 h incubation. However, NAC was more sufficient after 72 h incubation than gliclazide (Figure).
Conclusion: Our findings indicate that gliclazide may prevent cells from cytotoxic effect of hydrogen peroxide by decreasing the generation of ROS. These results support previous observations suggesting protective effect of this antidiabetic drug on beta-cells loss. However, further studies are needed to evaluate the mechanism(s) of protective effects of gliclazide against oxidative stress induced apoptosis.

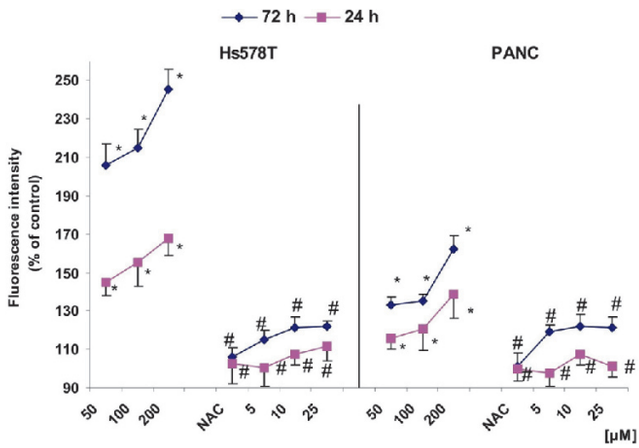

Figure. Induction of ROS by H2O2 in Hs578T and PANC cells in the presence and absence of antioxidant-NAC or gliclazide. The cells (10^4), seeded into 96-well microplates 24 h before the experiment, were treated with different H2O2 concentrations for 24 h or 72. Oxidation of 5 μM DCFH2-DA fluorescence probe was used for monitoring the produced ROS after drug treatment. In experiments with antioxidant or gliclazide, cells treated with 200 μM of H2O2 were preincubated with 3 mM NAC or 5-25 μM gliclazide for 1 h, then H2O2 was added and incubation was continued for another 24 h or 72 h. The results represent mean ± SD of four independent experiments. *P<0.05 in comparison to respective control cells taken as 100%, #P<0.05 indicates significant differences between H2O2-treated cells and samples preincubated with NAC or gliclazide.
Supported by: Medical University of Lodz 503 -0077-9

PS 83 Natural history of type 2 diabetes mellitus management

906

Uncontrolled type 2 diabetes treated with oral hypoglycaemic agents (OHA): therapeutic behaviour in primary care in France

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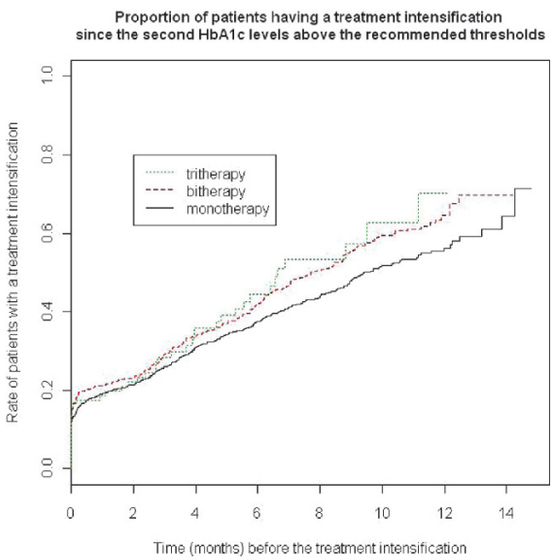
Background and aims: Current diabetes guidelines recommend that treatment should be intensified according to the HbA1c level. In France, HbA1c thresholds vary according to treatment patterns: mono-, bi- or tritherapy with OHA or with insulin. Recent epidemiologic data have confirmed that intensified therapies are still underused. The study objectives were: to identify the proportion of type 2 diabetic (T2D) patients with intensified treatment among those who needed such intensification according to HbA1c levels, to measure the time to intensification, to identify the factors related with this delay.
Materials and methods: A retrospective analysis was performed on a cohort of T2D patients from a large computerised longitudinal database in primary care. Patients analyzed were adult T2D patients, treated with OHA without insulin therapy or GLP-1 mimetic. Time to treatment intensification was measured from the last of two successive HbA1c levels above the recommended thresholds in France (6.5% for patients treated with a monotherapy, 7.0% for bitherapy and 8.0% for tri and quadritherapy). Treatment intensification was defined as an increase in the number of drug classes or an increased dose of the class being used or the introduction of insulin therapy or GLP-1 mimetic. Switches to a different class were not considered. Predictive factors were identified by Cox regression multivariate models.
Results: In the overall sample of 17,493 patients treated with OHA without insulin therapy or GLP-1 mimetic, 3,118 (18%) were identified as requiring a treatment intensification. Such intensification was performed respectively for 39% and 60% of those patients within a 6-month period and within a one-year period following the date of the second HbA1c value: 38% and 56% for patients treated with a monotherapy, 42% and 65% for patients treated with a bitherapy and 44% and 70% treated with a tritherapy. The rate of treatment intensification over time is positively correlated with HbA1c levels and with OHA treatment (bi- and tritherapy as compared to monotherapy) and negatively correlated with the age of the patient. No other explanatory variables related to the patients or to the physicians were identified.
Conclusion: With regard to current guidelines in T2D France, treatment intensification is still underused in primary care in France. Adherence to the guidelines appears to be mainly restrained by the age of the patient, with older patients being prescribed less frequently intensive treatment. Future guidelines should consider those points.



907

Patterns of use of glucose lowering treatments at baseline and during follow-up in ADVANCE

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Background and aims: The ADVANCE and UKPDS trials are the only two large scale glucose lowering trials that have separately reported major benefits on cardiovascular and renal outcomes. Both used glucose lowering regimens that were based on sulphonylureas with addition of other drugs as required. In the present analyses we examine the patterns of use of various glucose lowering treatments at entry to ADVANCE and during follow-up.
Materials and methods: The treatment patterns applied to all 11,140 patients randomized to receive either gliclazide modified release (MR)-based intensive glucose control, targeting a mean glycated hemoglobin (HbA1c) of <=6.5% (n=5,571), or standard guideline based glucose control (n=5569) were analyzed both at baseline and during follow-up.
Results: At baseline, 9% of patients were managed with diet and lifestyle alone, 43% were receiving a single oral glucose lowering agent, 42% were receiving two oral agents and 6% were receiving three or more oral agents. 72% were on a sulphonylurea, 61% on metformin, and only 4%, 9% and 2% on thiazolidinediones, acarbose and glinides respectively. Of the patients on monotherapy at baseline, 59% and 38% were on sulphonylureas and metformin respectively. The median duration of follow up was 5.0 years. In the group assigned intensive glucose control, the HbA1c fell gradually to the target of <=6.5% after 36 months and was maintained till the end of the study. By the end of follow-up, 91% of patients in the intensive control group were still on gliclazide MR (average daily dose of 103mg/day) and 59% of patients in the standard control group were on other sulphonylureas. At that time, 74% and 67% of patients were taking metforminin the intensive and standard glucose control groups respectively. Less than half of the subjects in the intensive control group required insulin by the end of follow-up (40%). The average time to the introduction of insulin in the intensive group was 44 months, considerably later than the 36 months taken to achieve the HbAlc target of 6.5%.
Conclusion: The gliclazide MR-based intensive glucose control regimen achieved its target HbA1c of 6.5% within 3 years and maintained it to the end of 5 years of follow-up. At baseline, sulphonylureas were taken by 72% of all patients and at the end of follow-up by 91% of patients in the intensive glucose control group and by 59% of patients in the standard control group. Sulphonylureas continue to play a critically important role in glucose control and in vascular and renal protection for patients with type 2 diabetes, as demonstrated with gliclazide MR in ADVANCE.
Supported by: Servier

908

Reasons why UK general practitioners do not initiate antihyperglycaemic therapy in older and younger patients following diagnosis of type 2 diabetes

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Background and aims: Research has reported benefits for early treatment of type 2 diabetes (T2DM). However, delayed treatment has been found more commonly in older patients. This study compared characteristics of younger and older patients with diagnosed T2DM who were untreated with antihyperglycaemic agents (AHAs) for at least 6 months following diagnosis and assessed physicians' reasons for non-treatment.
Materials and methods: A survey was conducted in Nov 2009-Jan 2010 among 358 UK general practitioners. Physicians provided data from chart review on patients aged ≥18 years were untreated with any AHA for ≥6 months after diagnosis. Each physician also chose reasons for not initiating AHA therapy for their selected patients. Thirty six potential reasons were classified into 4 major categories: mild hyperglycaemia, factors related to AHA therapy, issues with co-morbidities and/or polypharmacy, and patient-related reasons.

All analyses were stratified by patient's age at the time of T2DM diagnosis (younger [<65 years] vs. older [≥65 years]). Group differences were assessed with a t-test for mean, rank test for median and χ^2 test for proportions.
Results: Of the 2,028 patients provided by the physicians, 1,023 were younger (mean age=51 years; mean most recent HbA_{1c}=6.8%; 61% males) and 1,005 were older (mean age=74 years; mean most recent HbA_{1c}=6.8%; 54% males). Compared to younger patients, older patients had a longer duration of T2DM (median 25 vs. 18 months), lower BMI (29 vs. 31 kg/m^2), and a higher prevalence of cardiovascular conditions (18% vs. 5%) and microvascular complications (15% vs. 4%) (all p<0.001). The proportion of patients with most recent HbA_{1c} ≥6.5% (UK treatment target <6.5%) did not differ significantly between older and younger patients (58% vs. 59%, respectively). The most commonly reported reason for not initiating an AHA by physicians was related to mild hyperglycaemia and was not different between groups (86% for older and 88% for younger patients). Compared to younger patients, factors related to AHAs (46% vs. 38%) and issues with co-morbidities/polypharmacy (33% vs. 19%), both including safety-related issues, were more commonly reported reasons for not initiating AHA therapy in older patients (all p<0.001). The reported patient-related reasons were not different between older (41%) and younger (43%) patients.
Conclusion: Among patients who were untreated with an AHA for ≥ 6 months following diagnosis of T2DM, nearly 60% had a most recent HbA_{1c} above the recommended goal <6.5%. Mild hyperglycaemia was the most reported reason by physicians for non-treatment in all patients, regardless of age. AHA-related factors, e.g., "may cause hypoglycaemia", were more frequently reported by physicians as reasons for non-treatment among older patients. Given older patients have higher prevalence of vascular complications, delay in diabetes treatment for this population may have greater health implications than for younger patients.
Supported by: Merck

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The likelihood of initiating insulin is increased in UK patients with newly diagnosed type 2 diabetes who received initial monotherapy with sulphonylurea compared with metformin
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Background and aims: Despite initial treatment with a single antihyperglycaemic agent (AHA), the addition of other AHAs including insulin is ultimately required in many patients. This study examined the association of initial oral AHA monotherapy choice with the likelihood of subsequent addition of insulin among patients with newly diagnosed type 2 diabetes (T2DM).
Materials and methods: This retrospective cohort study used a sample from the MediPlus database of general practitioners in the UK. Patients with newly diagnosed T2DM during 1992 to 2008 who were ≥30 years old at the first T2DM diagnosis and who received either metformin (MET) or sulphonylurea (SU) as initial monotherapy in 1992 or beyond were included. The follow-up period lasted to the end of 2008 or the patients' latest data available. The time from initial oral AHA monotherapy (MET or SU) to insulin initiation was estimated based on prescription records. An adjusted Cox proportional hazards regression was conducted to evaluate the likelihood of insulin addition.
Results: Of the 15,926 patients with newly diagnosed T2DM who initiated either MET or SU monotherapy, 65% initiated with MET and 35% with SU. Patients initiated with SU were older at initial monotherapy (mean age 67 vs. 61 years), had lower BMI (mean 28 vs. 33 kg/m^2), and had higher incidence of insulin addition (21% vs. 9%) and shorter time to insulin initiation (median 5,504 vs. 5,613 days) compared to those initiated with MET (all p<0.001). Adjusted for gender, age at and the year of initial monotherapy, BMI, selected co-morbidities, and other medication use, patients started with SU monotherapy were more likely to initiate insulin (adjusted hazard ratio: 1.77 [95% CI: 1.53, 2.06]; p<0.001) than those started with MET.
Conclusion: After adjustment for potential differences in patient profiles which may be associated with beta-cell dysfunction (i.e., lower weight and older age), patients with newly diagnosed T2DM in the UK who started with SU monotherapy tended to receive insulin significantly faster and more often compared with those who started with MET monotherapy.
Supported by: Merck

910

Time to add-on medication use for patients with type 2 diabetes who failed metformin monotherapy
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Background and aims: Add-on medication regimens are recommended if target glycaemic goals for patients with type 2 diabetes (T2DM) are not achieved or sustained after initial metformin monotherapy. However, it is not clear how long it takes for additional treatment to be used after metformin monotherapy failure in clinical practice. This study was undertaken to address this question.
Materials and methods: The study cohort was selected from a large US electronic medical record database from 1/1/1997 to 12/31/2008. Included subjects had to be aged ≥18 years with a diagnosis of T2DM who had HbA_{1c} ≥7.0% or at least two fasting blood glucose levels of ≥126mg/dL (7 mmol/L). Treatment failure was defined as HbA_{1c} ≥7% (index date) after metformin monotherapy for at least 6 months. Baseline data were extracted during 1 year prior to the index date. Time to add-on medication use was the time between the index date to the first add-on medication use during follow-up period and was evaluated for the overall cohort and for three index HbA_{1c} subgroups: 7-8%, 8-9%, and >9%. A Cox proportional hazard model was employed to determine baseline clinical and demographic characteristics associated with shorter time to add-on medication use.
Results: There were 12,566 patients meeting the inclusion criteria; 8656, 2175 and 1735 had index HbA_{1c} 7-8%, 8-9%, and >9%, respectively. The overall mean (SD) age was 63 (12) years and 51% were female. The median time to add-on medication use was 15.7 months overall and 17.0, 13.9 and 11.3 months for patients with index HbA_{1c} 7-8%, 8-9%, and >9%, respectively. Higher index HbA_{1c}, greater body mass index, higher Charlson comorbidity index, younger age, males, lower LDL-cholesterol were significantly associated with shorter time to add-on medication use (all p<0.05).
Conclusion: In clinical practice in the US, it takes nearly 16 months for T2DM patients with sub-optimal glycemic level after initial metformin monotherapy to receive additional antihyperglycaemic therapies. There is room for improvement through disease management so that patients who fail metformin monotherapy and are eligible and appropriate for treatment intensification, receive add-on therapy sooner rather than later.
Supported by: Merck

911

Is three months sufficient to assess HbA_{1c} reduction in high-baseline patients? An analysis of HbA_{1c} time course after initiation of metformin alone or in combination with sitagliptin
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Merck Research Laboratories, Rahway, USA.

Background and aims: The ADA/EASD consensus algorithm for treatment of type 2 diabetes recommends that antihyperglycemic (AHA) medications be added if target A1C levels are not achieved within 2-3 months. We examined whether this timeframe is adequate to assess the full response to a new course of therapy.
Materials and methods: The time course of change in A1C was analyzed in data pooled from 2 randomized, double-blind, multicenter studies of drug-naïve patients treated for 30-31 weeks with metformin (MET) 2000 mg/day (uptitrated during Weeks 0-4) as monotherapy (N=803) or in combination with sitagliptin 100 mg/day (SITA/MET) (N=807). The analysis determined each patient's maximum A1C reduction ($\Delta A1C_{MAX}$) and the cumulative proportions of patients first achieving 95% of $\Delta A1C_{MAX}$ at Weeks 6, 12, 18, and 30. It included all patients who completed treatment without rescue (n=514 and 538 in the MET and SITA/MET groups, respectively) and 3 subgroups composed of patients with baseline A1C ≤8.0% (n=253), >8.0-≤9.5% (n=376), and >9.5 % (n=423).
Results: Mean $\Delta A1C_{MAX}$ was −2.2% and −2.6% in the overall MET and SITA/MET treatment groups. Both groups had significant reductions in A1C at the earliest time point measured. At Week 12, however, only 32.7% and 29.9% of patients on MET and SITA/MET, respectively, had reached 95% of $\Delta A1C_{MAX}$; at Week 18, the proportions were 71.4% and 73.0%, respectively. Evaluation after 12 weeks appeared to be especially premature in high-baseline A1C patients who received SITA/MET and had the greatest reductions in A1C

during treatment. Among SITA/MET-treated patients in the subgroup with baseline A1C>9.5% (n=223; mean $\Delta A1C_{MAX}$ = −3.7%), only 22.9% reached 95% of $\Delta A1C_{MAX}$ by Week 12, compared with 40.7% in the baseline A1C≤8% subgroup (n=123; mean $\Delta A1C_{MAX}$ = −1.2%).
Conclusion: This analysis found that full A1C-lowering efficacy of AHA medications was not demonstrated in most patients after only 3 months of treatment. Further, these findings suggest that patients with higher baseline A1C levels may require longer periods of time to reach a steady-state A1C level.

912

Discontinuing SU and initiating insulin detemir + sitagliptin: improved efficacy and similar safety vs. adding sitagliptin to a prior SU regimen. A TRANSITION™ trial subanalysis

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Background: Once-daily insulin detemir (IDet) is often prescribed for patients with T2D as add-on to OAD. IDet has not previously been evaluated in combination with a newer class of OAD, dipeptidyl peptidase-4 (DPP-4) inhibitors, which reduce enzymatic degradation of the incretin glucagon-like peptide-1 (GLP-1) and thereby enhance glucose-dependent insulin secretion. In this randomized, open-label, parallel group, 26-week trial of insulin-naïve T2D patients who were poorly controlled by a previous regimen with metformin (Met) ± sulphonylurea (SU) or other OADs, IDet was administered in combination with the DPP-4 inhibitor sitagliptin (SITA) and Met with prior SU, if any, discontinued. This was compared for efficacy and safety against SITA + Met in combination with the subjects' prior SU regimens, if any, continued. This is a sub-group analysis which reports outcomes only for patients with pre-trial SU treatment regimens.
Materials and methods: This subgroup analysis is comprised of the 75% of subjects (*n*=80) in the IDet + SITA group and the 77% of subjects (*n*=85) in the SITA ± SU group who had a pre-trial SU regimen (SITA + SU). Treatment groups compared IDet + SITA as add-on to Met, coupled with discontinuation of prior SU therapy, vs. SITA as add-on to Met + pre-trial SU. Results described herein are after 26 weeks' treatment with once-daily IDet + SITA (100 mg QD) + Met (≥1000 mg) (IDet/SITA) with discontinuation of pre-trial SU, vs. SITA + Met + SU (SU/SITA) (at each subject's pretrial SU dose). A normal linear regression model was used to analyze HbA_{1c}, FPG, body weight and BMI.
Results: Observed baseline A1c was 8.46 and 8.58%, with final mean estimated A1c of 7.23 and 7.63% for the IDet/SITA and SU/SITA groups, respectively. Estimated reductions in A1c, after baseline adjustment for covariates, were 1.29 and 0.88% (est. mean diff. = -0.40%; 95% CI = [-0.66; -0.15], *p*=0.002), for IDet/SITA and SU/SITA, respectively. FPG fell to 6.20 and 8.53 mmol/L from observed baselines of 9.61 vs. 9.79 mmol/L for the IDet/SITA and SU/SITA groups, respectively, with estimated reductions of 3.51 vs. 1.18 mmol/L (est. mean diff. = -2.34 mmol/L, 95% CI = [-3.00;-1.68], *p*<0.001). Body weight and BMI decreased in both subgroup treatment arms with no significant differences between groups (-1.10 vs. -1.42 kg, and -0.40 vs. -0.52 kg/m^2, *p*=NS, IDet/SITA vs. SU/SITA, respectively). No major hypoglycemia was reported. Minor hypoglycemia (PG <3.1 mmol/L) was low in both arms (0.58 and 0.70 estimated events/patient-year, Adjusted Rate Ratio = 0.84 [95% CI: 0.27, 2.65]; *p*=NS, IDet/SITA vs. SU/SITA, respectively). Final insulin dose was 0.59 U/kg at trial end.
Conclusion: The results of this subgroup analysis - improved A1c, FPG, low hypoglycemia and modest decreases in body weight and BMI in both arms - supports substitution of IDet/SITA for SU, or addition of SITA to SU + Met. Better glycemic control (i.e. lower A1c and FPG) was achieved for IDet/SITA compared to the SU/SITA arm among these pre-trial SU users with low incidence of hypoglycemia. Therefore, discontinuation of SU and substitution of once-daily IDet in combination with a DPP-4 inhibitor, SITA and Met is a safe and more effective treatment option for insulin-naïve T2D patients, compared to adding SITA to an existing SU and Met regimen.
Supported by: Novo Nordisk

913

Long-term patterns of statin therapy in patients with type 2 diabetes mellitus compared to long-term oral anti-diabetic medication patterns

E.J.F. Lamberts[1], M.L. Bouvy[1], P.C. Souverein[1], G. Nijpels[2], L.M.C. Welschen[2], J.G. Hugtenburg[2], J.M. Dekker[2];
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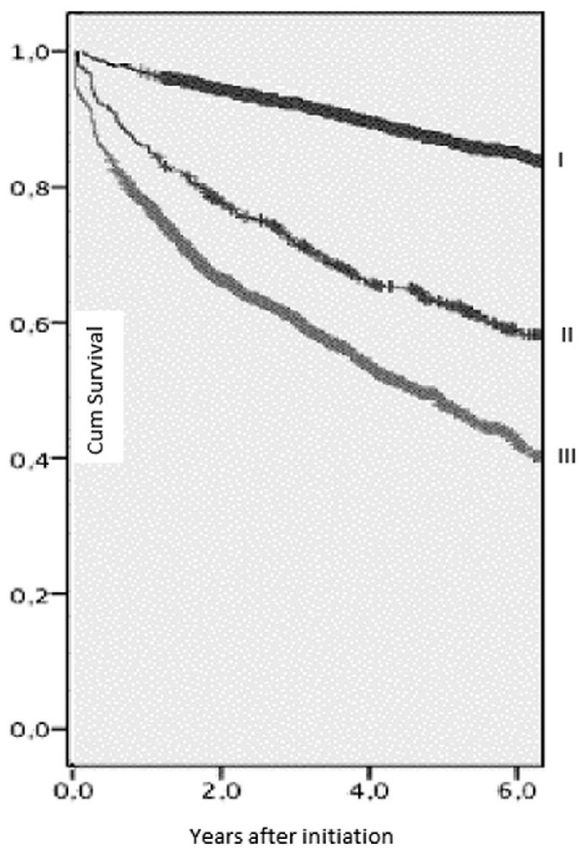
Background and aims: Statins are effective in the prevention of cardiovascular disease in type 2 diabetes mellitus. However studies on long-term use of statins among type 2 diabetes mellitus patients are scarce. The aim of this study was to describe long-term patterns of statin use among patients with type 2 diabetes mellitus and compare discontinuation of statins and oral antidiabetics.
Methods: A cohort study among 2072 patients with type 2 diabetes mellitus who initiated treatment with statins between 1999 and 2007. Drug dispensing data were extracted from 17 community pharmacies in a geographically well-defined region in The Netherlands. Patients were classified as using statins before (prevalent users) or after (incident users) the initiation of oral antidiabetics. Patients were considered to have discontinued statin therapy when an interval of 180 days or more occurred between the theoretical end date of a statin prescription and a subsequent statin prescription. This was done in the same way for oral antidiabetics in order to compare discontinuation between the drug groups.
Results: The proportion of patients with type 2 diabetes mellitus using statins increased between 1999 and 2007. Discontinuation rates for statins were higher compared to discontinuation of oral antidiabetic drugs (52.1 vs.15.0%). Moreover, incident statin users were more likely to discontinue statin therapy compared to prevalent statin users (62.8 vs. 48.2%).
Conclusions: Although statins are increasingly prescribed to patients with type 2 diabetes mellitus, discontinuation of statins is high compared to discontinuation of antidiabetics. This could result in suboptimal therapeutic outcomes for patients with type 2 diabetes mellitus.



Long-term continuation of statins and oral anti-diabetic drugs. Shown for anti-diabetics (I), prevalent statin users (II) and incident statin users (III). (Log Rank p < 0.0001)

Supported by: St. MAG

PS 84 "Metabolic syndrome": definition and management

914

Remission of the metabolic syndrome three years after screening for increased waist circumference

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Background and aims: Screening for cardiovascular risk factors and diabetes among overweight and obese individuals might be an attractive option with potential health benefits. In 2006, such a screening in primary care was performed to detect individuals with the metabolic syndrome (MetS) by letting them measure their waist circumference at home as a first step. Among individuals aged 20-70 years, previously not known with hypertension, diabetes or dyslipidemia and selected by means of a self-measured increased waist circumference, 473 new MetS cases were detected. They were advised to contact their general practitioner. However, screening of individuals with a high cardiovascular risk is only meaningful if adequate subsequent and effective action is undertaken. The aim of this study was to assess the remission of the MetS three years after screening followed by usual care in general practice.
Materials and methods: 432 individuals with screen-detected MetS (those of the original group of 473 patients that still visited the same general practice) were invited for follow-up measurements, which included a physical examination and laboratory tests. The MetS was defined according to the NCEP ATP III criteria. We also invited a random selection of 280 individuals who had an increased self-measured waist circumference during screening, but did not meet the MetS criteria at that time.
Results: The overall response rate was 84%. 63% of the responders indicated to be interested in follow-up measurements (the 'participants'). 197 individuals with MetS at screening and 179 individuals without MetS at screening underwent all follow-up measurements. A significant improvement in all MetS components, except for glucose, was seen in the group with screen-detected MetS (table). The remission rate was 53%. The only significant changes in the group without MetS in 2006 were a decrease in diastolic blood pressure, an increase in triglyceride level and a decrease in HDL-cholesterol level in women. 15% of the participants in this group developed the MetS at follow-up. Non-participants and participants were comparable in age, gender and mean level of MetS components in 2006. The 16% non-responders with screen-detected MetS were significantly younger than the responders (both participants and non-participants) with screen detected MetS.
Conclusion: Screening for MetS among overweight and obese individuals, followed by care as usual, leads to significant improvements in all MetS components and a remission rate of 53% among the individuals with screen-detected MetS. This might be an attractive option with potential health benefits.

Mean levels (SD) of MetS components, BMI and weight in 2006 (screening) and 2009 (follow-up)

	2006	2009	P-value
BMI (kg/m2)	30.2 (3.6)	29.3 (4.1)	<0.001
Weight (kg)	93.2 (15.2)	90.8 (16.1)	<0.001
Waist circumference men (cm)	109.9 (7.4)	106.1 (10.0)	<0.001
Waist circumference women (cm)	99.6 (8.8)	96.1 (10.7)	<0.001
Systolic blood pressure (mmHg)	143.7 (15.0)	135.5 (13.5)	<0.001
Diastolic blood pressure (mmHg)	88.0 (7.5)	82.4 (7.7)	<0.001
Triglycerides (mmol/L)	2.2 (1.1)	1.9 (0.9)	0.001
HDL-cholesterol men(mmol/L)	1.1 (0.3)	1.2 (0.3)	<0.001
HDL-cholesterol women (mmol/L)	1.3 (0.3)	1.4 (0.3)	<0.001
Fasting blood glucose (mmol/L)	5.3 (1.2)	5.4 (0.8)	0.02

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Improving cardiometabolic risk by lifestyle modification in viscerally obese men: is weight loss the best therapeutic target?

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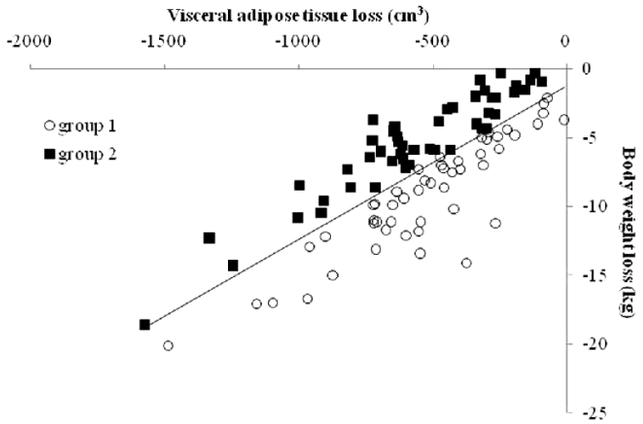
Background and aims: Visceral obesity is associated with a diabetogenic/atherogenic cardiometabolic risk (CMR) profile. Most intervention studies aiming to reduce CMR have used magnitude of weight loss as their main outcome measure. The present work compared the CMR profile response to a one-year healthy eating/physical activity lifestyle modification program of high risk viscerally obese men (waist girth ≥ 90 cm, triglycerides ≥ 1.69 mmol/L and/or HDL cholesterol < 1.03 mmol/L) classified into two groups according to both weight loss and loss of visceral adipose tissue (VAT).
Materials and methods: From the 144 viscerally obese men who participated in the study (age 47.5 ± 9.0 years, waist girth 107.8 ± 8.5 cm, triglycerides 2.52 ± 0.89 mmol/L, HDL-cholesterol 0.95 ± 0.16 mmol/L), 109 completed the first year of intervention. In the present analysis, we examined the relationship between weight loss and loss of VAT in the subgroup of 100 men who lost both weight and VAT. Body weight, body composition and fat distribution were assessed by anthropometry and DEXA/computed tomography. Cardiorespiratory fitness and fasting lipoprotein/lipid profile were assessed, and an oral glucose tolerance test (75 g) was performed. The regression line between weight loss and loss of VAT was used to define two groups of men, below and above the regression line (figure). Group 1 and group 2 were defined as men having lost more (group 1: 10 ± 4 %) or less (group 2: 6 ± 4 %) body weight than predicted by the regression line.
Results: At baseline, group 1 presented higher body weight and subcutaneous adipose tissue (SAT) volume than group 2 (97 ± 13 vs. 92 ± 9 kg and 1886 ± 558 vs. 1585 ± 476 cm^3, for group 1 and 2 respectively, $p < 0.05$), but lower VAT volume (1843 ± 493 vs. 2043 ± 431 cm^3, respectively, $p = 0.03$). After the one-year intervention, group 1 had lost more weight and SAT than group 2 (-9.2 ± 4.1 vs. -5.2 ± 3.9 kg and -446 ± 284 vs. -245 ± 195 cm^3, respectively, $p < 0.0001$). However, loss of VAT was similar between the two groups. Finally, despite greater absolute and relative losses of body weight and body fat in group 1 than 2, both groups showed essentially similar improvements in their CMR profile.
Conclusion: Provided that loss of visceral AT is similar, magnitude of weight loss does not appear to have any additional effects on the response of the CMR profile to a one-year lifestyle modification program. Therefore, our results suggest that magnitude of VAT loss may be a better therapeutic target than weight loss.



Supported by: the CIHR.

916

Optimal serum alanine aminotransferase cutoffs for the metabolic syndrome in Chinese

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is the most frequent liver disease in China and western countries. It has been demonstrated that NAFLD was significantly correlated with all components of the metabolic syndrome (MetS), and could independently predict the risks of type 2 diabetes and coronary vascular disease (CVD). Serum alanine aminotransferase(ALT) concentration is the most commonly measured variable for assessment of liver disease. However, the current ALT upper limit (40 IU/L) often fails to identify patients with MetS and potential hepatic fat infiltration, thus underestimating the risks of type 2 diabetes and CVD. It has been recently recommended that the normal upper limit for ALT should be revised to 30 IU/L for men and 19 IU/L for women to identify NAFLD or chronic HCV infection in western countries. However, the optimal ALT cutoffs for metabolic syndrome in Chinese communities were poorly studied. The aim of the present study was to determine the optimal cutoffs for ALT linking to the risk of the MetS in Chinese subjects.
Materials and methods: The study population consisted of 440 subjects (242 male and 198 female) aged from 18 to 80 years recruited from the local community and outpatient department of endocrinology, Shanghai Zhongshan Hospital. Participants with hepatitis B or C, excessive alcohol intake and other hepatic disease were excluded. A standard interview, anthropometrics (height, weight, waist, hip circumference and blood pressure), Laboratory parameters (ALT, serum lipid, fasting plasma glucose, 2 h postload plasma glucose) were conducted for each participant. Metabolic syndrome was defined according to the International Diabetes Federation criteria. Statistical analyses were performed with SPSS for windows 15.0. All reported p values were two-tailed and *p*-values less than 0.05 were considered statistically significant. Univariate analysis of variance was used to detect the association between ALT level and all MetS components. Receive operating characteristic (ROC) curve analyses were utilized to determine the appropriate cutoffs of ALT for identifying individuals with MetS.
Results: In 242 men there were 97(40.1%) with MetS, and in 198 women there were 89(55.1%) with MetS. The frequency of MetS was elevated with increased ALT level in both male and female participants. Stratified according to sex, Univariate analysis showed ALT level was associated with waist circumference, serum cholesterol and LDL-c in female, but only associated with waist circumference in male. The optimal cutoffs were 36IU/L in male and 19IU/L in female for ALT to identify MetS patients. Compared with the current ALT upper limit, the revised ALT cutoffs were more sensitive and efficacious in identifying MetS, especially in women (Table 1).
Conclusion: The optimal cutoffs of ALT for MetS are 36IU/L for men and 19IU/L for women in Chinese. The current used ALT upper limit (40 IU/L) could underestimate MetS accompanied by NAFLD, especially in women.

Table 1 Sensitivity and specificity of ALT to determine subjects with MetS based on the IDF definition

	Female(n=198)		Male(n=242)	
	Optimal ALT cutoff	Current ALT cutoff	Optimal ALT cutoff	Current ALT cutoff
Area under curve	0.673		0.681	
cutoff value	19U/L	40U/L	36U/L	40U/L
Sensitivity	0.764	0.371	0.691	0.65
Specificity	0.587	0.798	0.646	0.667
PPV(%)	0.602	0.6	0.563	0.563
NPV(%)	0.753	0.608	0.76	0.742
J value*	0.351	0.169	0.337	0.316

IDF, International Diabetes Federation; MetS, metabolic syndrome; NPV, negative predictive value; PPV, positive predictive value; ALT, alanine aminotransferase; J value, Youden Index= sensitivity + specificity-1
*The optimal cutoffs of ALT were obtained when the Youden Index was maximal.

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917

Two major patterns of dynamics in adipokine serum concentrations over 2 years of dietary weight-loss intervention

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Background and aims: During dietary interventions, adipokines' serum concentrations may be affected by changes in adipose tissue mass and/or by direct effects of healthier dieting. However, which are the adipokines that follow hormones and lipid parameters that mainly reflect weight changes, versus those that are primarily affected by the dietary change that could persist beyond weight change is largely unknown.
Materials and methods: In the 2-year Dietary Intervention Randomized Controlled Trial (DIRECT) among patients with type 2 diabetes (T2D, n=45) and non-diabetics (n=277), which have been randomly assigned to one of three diets: low-fat, Mediterranean, or low-carbohydrate, we analyzed the dynamics in circulating levels of adipokines, as well as serum concentrations of traditional biomarkers, and their association with 2-year changes in homeostasis model assessment-insulin resistance (HOMA-IR) and carotid artery intima-media thickness (IMT).
Results: Both qualitative analysis of the changes in biomarker levels at baseline, 6 and 24 months of intervention, and utilizing a non-biased mathematical modeling approach, revealed 2 major patterns of dynamics. Pattern A included biomarkers whose dynamics closely reflect changes in body weight, which exhibited a rapid decline (0-6 months) followed by weight stabilization or regain (7-24 months). This pattern included fasting circulating levels of insulin, triglycerides (TG), leptin, chemerin, monocyte chemoattractant protein-1 (MCP-1), and retinol binding protein-4 (RBP4). Pattern B consisted of biomarkers that displayed a continuing, cumulative increase or decrease throughout the 24 months of dietary intervention, despite the partial regain in mean body weight. This group included high molecular weight (HMW) adiponectin, HDL-cholesterol, CRP, fetuin-A, progranulin, and vaspin. Patterns A and B were similar among patients with T2D and non-diabetics. In models adjusted for age, sex and assigned diet group, a greater decline in either chemerin (beta=0.136, p=0.034) or leptin (beta=0.250, p<0.001) within the first 6 months of intervention were associated with lower levels of HOMA-IR at 24 months. In multivariate models adjusted for age, sex, assigned diet group, and 24 months changes in weight, chemerin, progranulin and MCP-1, greater decrease in fetuin-A predicted a larger decline in IMT of the carotid artery by the end of the 2 years intervention (beta = 0.201, p=0.040).
Conclusion: During a 2-year dietary intervention, leptin, chemerin, MCP-1 and RBP4 corresponded mainly to body weight, similar to serum insulin and TG levels. In contrast, HMW adiponectin, fetuin-A, progranulin, and vaspin exhibited cumulative improvement despite partial weight regain, similar to the changes in HDL-cholesterol and CRP. The two patterns underscore weight -associated versus healthy diet -induced beneficial effects beyond weight loss, and suggest a potential predictive value for clinically-relevant metabolic and cardiovascular endpoints.
Supported by: Israeli Ministry of Health, DFG, AF

918

Effect of dietary fat modification on insulin secretion in subjects with the metabolic syndrome

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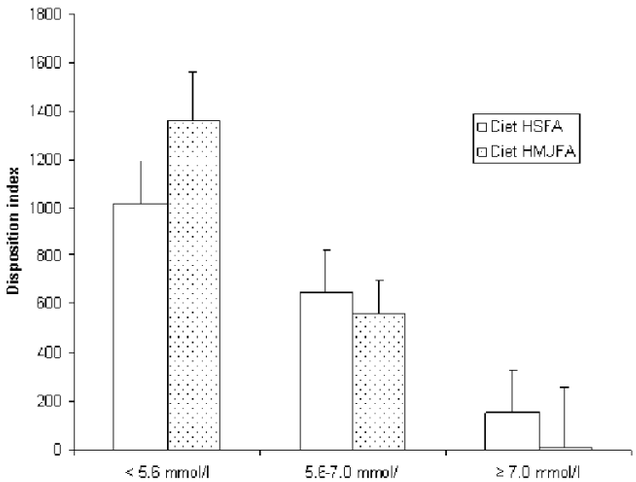
Background and aims: Both β-cell dysfunction and insulin resistance contribute to the development of type 2 diabetes. Dietary fat may have beneficial effects on insulin sensitivity, but the effect on insulin secretion is less clear. We investigated the effect of dietary fat modification on insulin secretion in European subjects with the metabolic syndrome.

Material and methods: In a 12 weeks parallel, randomized controlled dietary intervention trial (LIPGENE) 486 subjects were randomly assigned to isoenergetic diets: High fat (38 energy%) diets rich in saturated fat (HSFA) or monounsaturated fat (HMUFA) or low-fat (28 energy%), high complex carbohydrate diets with (LFHCC n-3) and without (LFHCC-control) 1.2 g/day of n-3 PUFA. The β-cell function was measured as acute insulin response (AIRg) and disposition index (DI), modeled from intravenous glucose tolerance test (IVGTT). The mean age was 54.4 ± 9.0 years, BMI was 32.3 ± 4.1 kg/m2 and 45% were males.
Results: There was no overall effect on AIRg and DI of the dietary intervention, but there were significant diet*fasting category of glucose interactions for AIRg (p=0.021) and DI (p=0.001). In subjects with normal fasting glucose and preserved first phase insulin secretion the effects of dietary intervention were significantly different between the HMUFA and the HSFA diets for both AIRg (p=0.015) and DI (p=0.010). There were no differences between the LFHCC diets.
Conclusion: The effects of dietary fat modification on β-cell function were minor in the total cohort, but in normoglycemic subjects the HMUFA diet improved AIRg and DI as compared to the HSFA diet.



Supported by: EU 6FP

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Defining waist circumference cut points for South Asians in the United Kingdom using measures of dysglycaemia

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Background and aims: To determine waist circumference cut-points in South Asians giving equivalent levels of dysglycaemia as in white Europeans with waist levels equivalent to obese BMI cut-points.
Materials and methods: 4688 white Europeans and 1353 South Asians aged 40- 75 years were screened for Type 2 diabetes. Regression models for fasting glucose, two-hour post-challenge glucose, HbA1c and a glucose factor (derived using principal components from the three glucose parameters), adjusted for centred age and stratified by sex, were used to identify waist circumference cut points in South Asians that correspond to glycaemic values at the recommended 'obese' waist circumferences of 102cm for males and 88cm for females in white Europeans.
Results: For South Asians the derived waist circumference cut points were substantially lower than for white European males and females across all glucose parameters. An overall glucose factor score at a cut point of 102cm in white Europeans was met by South Asian males at a waist circumference of 84cm. Similarly, a cut point of 88cm for white European females was equivalent to cut point 69cm for South Asian females. Here 7.5% of South Asian males have a waist circumference ≤84cm and 4.2% of females have a waist circumference ≤69cm.
Conclusion: Remarkably low cut points for defining very high waist circumferences for males and females are needed in South Asians for detecting equivalent levels of dysglycaemia as in obese white Europeans. Although the IDF already accepts a lower cut point for South Asian males (90cm), the even lower cut points suggested herein suggest that South Asian people should be considered at high risk of diabetes irrespective of waist circumference measurements.
Supported by: LNR CLARHC

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Assessing cardiometabolic risk among shift workers

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Background and aims: The term of cardiometabolic risk includes a cluster of cardiovascular risks beyond the metabolic syndrome. Shift workers, possibly due to impairment in circadian biological rhythm, may be at higher cardiometabolic risk. In order to assess cardiometabolic risk in shift workers, a cross-sectional study was performed among active workers (aged 25-66 years, with a minimal shift working experience of 5 years).
Materials and methods: We investigated 481 workers (121 male, 360 female) in our study, most of them were employees in light industry (58.2%) or in public service (23.9%). At enrolment, past medical history was recorded and anthropometric measurements and physical examination were performed in each subject. Validated questionnaires were used to characterize daily activity, eating and smoking habits. Fasting venous blood sample was taken for measuring laboratory parameters. Data from shift workers (n=234, 54 men and 180 women, age 43.9±8.1 years) were compared to those of day workers (n=247, 67 men and 180 women, age: 42.8±8.5 years).
Results: Weight (76.6±16.1 *vs* 73.9±17.6 kg; p<0.05), BMI index (27.5±5.3 *vs* 26.0±4.9 kg/m^2; p<0.01), systolic blood pressure (123±19 *vs* 119±16 mmHg, p<0.01), the prevalence rate of diabetes (4.3 *vs* 1.2 %; p<0.05) and cardiovascular diseases (3.8 *vs* 0.8 %; p<0.05) in the past medical history were higher in shift workers as compared to day workers. In addition, proportion of subjects with regular physical activity in leisure time were lower (20.6 *vs* 38.7 %; p<0.001) and that of current smokers were higher (35.0 % *vs* 19.5 %; p<0.001) in shift workers than in day workers. As for laboratory findings, HDL-cholesterol level was lower in female shift workers than in female day workers (1.56±0.32 *vs* 1.68±0,36 mmol/l; p<0.01).
Conclusion: These data indicate that middle-aged, active shift workers, as compared to day workers, are at higher cardiometabolic risk. Thus, our study highlights the importance of measures for preventing cardiovascular diseases in shift workers.
Supported by: Hungarian Diabetes Association

921

Immunological and cardiometabolic risk scores in the prediction of incident type 2 diabetes and coronary events: MONICA/KORA Augsburg case-cohort study

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Background and aims: Systemic concentrations of acute-phase proteins, cytokines, chemokines and soluble adhesion molecules are associated with the risk of type 2 diabetes and coronary events. The association of each of these biomarkers alone with incident disease is too weak for the prediction of outcomes, but the predictive value of combinations of multiple inflammation-related biomarkers is still unclear. This study aims to address the following questions: (i) what is the predictive value of inflammation-related biomarkers for incident type 2 diabetes and coronary events, (ii) are these predictive values comparable with the those of established biomarkers of cardiometabolic risk, and (iii) can the predictive value be improved by combining both sets of risk factors?
Materials and methods: The study investigates inflammation-related biomarkers (measured in non-fasting serum samples) and additional cardiometabolic risk factors in a prospective case-cohort study within the population-based MONICA/KORA Augsburg cohort. Analyses with the endpoint type 2 diabetes are based on 436 individuals with and 1413 individuals without incident type 2 diabetes. Analyses for coronary events are based on 314 individuals with and 1662 individuals without incident coronary events. The follow-up was 10±5 years. Receiver operator characteristic (ROC) analyses were used to calculate areas under the ROC curve (AROC) for different sets

of risk factors for both incident type 2 diabetes and incident coronary events: (a) basic model: adjusted for age, sex, and survey; (b): immunological model: factors from (a) plus CRP, IL-6, IL-18, MIF, TGF-beta1, MCP-1, IL-8, IP-10, RANTES, adiponectin, leptin, sE-selectin, sICAM-1; (c) cardiometabolic model: factors from (a) plus BMI, systolic blood pressure, total cholesterol/HDL cholesterol ratio, parental history of diabetes or myocardial infarction (according to the respective endpoint), smoking, alcohol, physical activity; (d) full model: combination of risk factors in (b) and (c).
Results: For the prediction of type 2 diabetes, the AROC for the basic model was 0.733. Addition of either inflammation-related biomarkers (as continuous variables) or cardiometabolic risk factors resulted in an increase to 0.803 in both models. A combination of all risk factors predicted type 2 diabetes with an AROC of 0.845. For the prediction of coronary events, the basic model had a higher predictive value (AROC 0.802), whereas the addition of inflammation-related or cardiometabolic risk factors led to less pronounced increases (AROC 0.825 and 0.836). The combination of all risk factors resulted in a similar predictive value as for type 2 diabetes (AROC 0.849).
Conclusion: The addition of multiple inflammation-related biomarkers to a basic prediction model and to a model including cardiometabolic risk factors increased the AROC in the prediction of type 2 diabetes and coronary events, although the increase was less pronounced for the latter endpoint. As limitation, it is important to note that the results were based on non-fasting blood samples and cannot be extrapolated to different study settings with more complete assessment of diabetes risk factors including glucose, insulin and HbA1c measurements.
Supported by: DFG

922

Significant type 2 diabetes incidence reduction in high risk participants, after three years of an intensive lifestyle intervention in primary care (DE-PLAN-CAT)
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Background and aims: Many Lifestyle interventions have demonstrated their efficacy preventing Type 2 Diabetes although none of them have shown their benefits into real clinical practice. Public health strategy on type2 diabetes prevention in primary health care. The European coordinated project DE-PLAN (*Diabetes in Europe-Prevention using Lifestyle, Physical Activity and Nutritional intervention*) evaluates its effectiveness in Primary Health Care.
Materials and methods: Public health strategy on type2 diabetes prevention in primary health care. Two-step multicentre cohort study: cross-over period (1y-screening) plus a follow-up period (3y-preventive intervention). In Catalonia (DE-PLAN-CAT) has 18 participating centres, more than 150 professionals (GP+nurses) and a representative randomized population based sample between 45-75 yo. Randomized non-invasive diabetes screening program by means of the FINDRISC score comparing with the oral glucose tolerance test (OGTT). All those high risk participants, without Diabetes, received a lifestyle intervention (self-acting informative intervention or intensive). Both groups were followed by "usual care" (self administered group) or by periodically motivation reinforcement. We performed an OGTT after 3 year of the intervention (An OGTT was yearly performed).
Results: A total of 2547 non-diabetic subjects > 45 y were contacted with a 80.6% positive response rate (n=2054). Of them 552 (26.9%) had high diabetes risk and 251 (45.5%) type 2 prediabetes. 210 (38%) were allotted to self-acting informative intervention and 342 (62%) to intensive lifestyle intervention. No differences in risk factor profile or in glycaemic status were detected. Mean annual drop-out rate was 11.4%, statistically higher in the informative group (14,4% / 9,6%; p<0,001). First year (n=476), 37 participants (7.8%) developed Diabetes, finishing the study. Second year (n=383) were diagnosed 32 new cases (8.4 %) and 21 (7.2%) in the third year (n=293). Cumulated diabetes incidence was 24.9%, 32,8% self-acting group vs 21% in the intensive intervention (p<0,01) [RRR 36%]. Progression rates (normality to prediabetes or diabetes and prediabetes to diabetes) were 40.3% and 31.7% (RR=21,3%). Regression rates (prediabetes to normality) were 5.9% and 13.6%, respectively (relative increase =130,5%).
Conclusion: Lifestyle intensive intervention has reduced significantly Type 2 Diabetes incidence, compared with a self-acting informative, in the Spanish Primary Health Care usual practice.
Supported by: DGSanco, FIS and Generalitat de Catalunya (Public Health)

923

Prenatal environmental exposures that may influence beta cell function or insulin sensitivity in middle age
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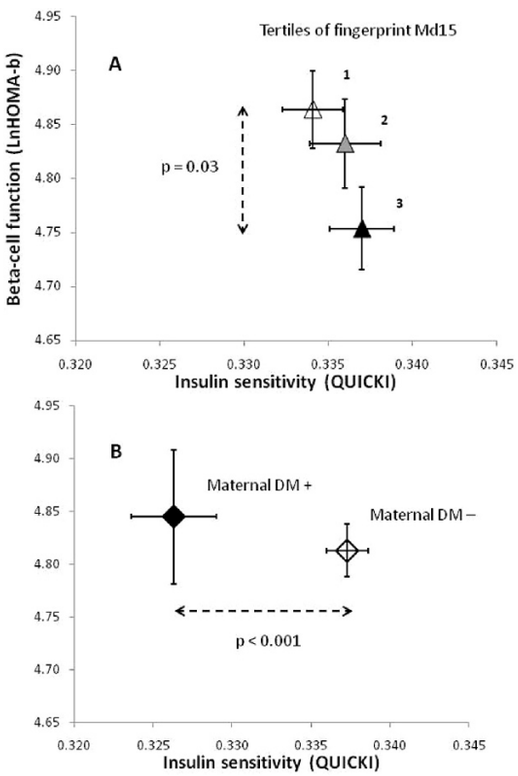
Background and aims: Patterns of fetal and childhood growth are associated with subsequent diabetes, but the underlying mechanisms remain unclear. Few studies have associated the early gestational environment with postnatal physiologic impairments to normal glucose metabolism. Fingerprints are permanently fixed in the first half of pregnancy, and increased values of a marker that contrasts fingerprint ridge counts between the thumbs and fifth fingers (Md15) have been linked to type 2 diabetes. Fingerprint Md15 has been associated also with seasonal features of the early prenatal environment. We studied adults metabolically to explore mechanisms explaining prenatal influences on insulin physiology in later life.
Materials and methods: Among 763 adults without known diabetes from the Dutch Hunger Winter Families Study we tested the associations of Md15 with homeostatic-assessment indices of pancreatic beta-cell function (HOMA-b) and insulin sensitivity (QUICKI). For either outcome index, linear regression analyses included terms for Md15 tertiles and for maternal history of diabetes as reported by each participant. All models were corrected for sibling pairs and adjusted for age, sex, and gestational and periconceptional famine exposures.
Results: Fingerprint Md15 was inversely associated with HOMA-b (p<0.05 for linear trend) but not with QUICKI. In contrast, a maternal history of diabetes was associated with decreased QUICKI (p<0.001) but not with HOMA-b. Paternal history of diabetes was not associated significantly with either index. Birth weight (available for 520 participants) was positively associated with increased QUICKI (p<0.05 for linear trend across tertiles) but not with HOMA-b.
Conclusion: Since Md15 describes variation in the anterior-posterior growth gradient of the early fetal hand, it is noteworthy that this permanent fingerprint characteristic is associated also with beta-cell function in later life. This finding appears consistent with rodent data on the role played by hedgehog signaling proteins in development of the fetal pancreas as well as the fetal forelimb. Research into the environmental circumstances associated with morphological features in the hand may suggest prenatal strategies for optimizing beta-cell function in adult life.



Supported by: NIH

PS 85 Diabetes in childhood

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Seasonal variation of type 1 diabetes incidence in childhood in Germany

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Background and aims: Seasonal variation of T1DM onset has been investigated in various studies and the results are conflicting. A sufficiently large cohort of cases is an important precondition for valid estimation of seasonal variation. Aim of the study was to investigate the seasonal variation of T1DM in children 0-14 years of age in the large risk population of the German federal state North Rhine-Westphalia (NRW) during the 12-year period 1996-2007.
Materials and methods: Data were taken from the NRW diabetes incidence register ascertaining newly diagnosed cases of T1DM by means of three data sources. During the study period a total of 7,128 newly diagnosed diabetic children aged 0-14 years (3,678 boys, 3,371 girls) were registered, the average risk population was 2.84 million children. The completeness of ascertainment was estimated to be 98%. Overall, sex- and age-specific (0-4, 5-9, 10-14 years) seasonal variation of the monthly T1DM incidence were analysed assuming a Poisson distribution of cases. The monthly expected mean of the incidence was modelled using a log-linear regression including a linear term for temporal trend and sinus and cosine terms for seasonal variation. The analyses were additionally adjusted for overdispersion of incidence data and, where appropriate, for sex and age at onset. Month of diagnosis was used as independent time variable. Seasonal variation was assumed to be constant over the study period.
Results: Overall, the average monthly incidence rates ranged between 16.6 per 100.000 person-years in July and 24.9 in January. Lower average incidences were found from April to August and higher incidences between September and March. According to the fitted sinusoidal regression model the seasonal variation of T1DM incidence was significant ($p<0.001$) with the trough at the end of June-July and the peak in December-January. The ratio of model-based maximum and minimum incidences was 1.34. Similar significant seasonal patterns ($p<0.001$) were observed among male and female patients, however, the amplitude was larger for males than for females (max/min-ratio: 1.46 vs. 1.24). Further, among males the trough was slightly earlier than among females. Significant seasonal variation was also detected for all age groups (0-4yrs: $p=0.005$; 5-9 and 10-14 yrs $p<0.001$). The magnitude of seasonal variation was similar in both older groups (max/min-ratio: 5-9 yrs: 1.43; 10-14 yrs: 1.38) but was considerably minor among the youngest children (max/min-ratio: 1.25). The model-based troughs in the age groups 0-4, 5-9 and 10-14 yrs were in May, June, and July, respectively.
Conclusion: This study based on a large cohort of T1DM cases showed significant seasonal variation in both sexes and all age groups. Seasonal patterns differed slightly between sexes and more distinctly between age groups. Seasonality of T1DM onset points to the importance of environmental factors in disease aetiology. Seasonal factors stressing beta cells (e.g. viral infections) may account for the variation. Delayed diagnosis in older children may account for the shift of the trough with age. Further research is needed to identify causes of the differing seasonal patterns between age groups.
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Seasonal variability of HbA_{1c} in children and adolescents with type 1 diabetes

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Background and aims: Hemoglobin A1c (HbA1c) is an useful measure of average glycemic control and a widely accepted marker of the risk of long-term microvascular complications in people with type 1 diabetes (T1DM). The aim of the study was to determine whether there is a seasonal variability in glycated hemoglobin levels.
Materials and methods: Inclusion criteria to the study were: T1DM with duration of at least 1 year and patient's age below 18 years at inclusion. Data from the laboratory database were cross-referenced with the clinical database application and verified manually by two independent researchers. During the analyzed period Feb-2006 to Oct-2009 a total of 6001 HbA1c results were recorded within the laboratory. HbA1c was measured by means of ion-exchange high-performance liquid chromatography using the Bio-Rad VARIANT Hemoglobin A1c Program (Bio-Rad Laboratories, USA). Trend and autocorrelation of residuals were analyzed.
Results: Out of 6001 available measurements, 5656 valid results were analyzed. Median HbA1c was 7.40% (25-75% range: 6.80-8.30%). The highest concentrations of HbA1c were observed in February, November and December, while August and September showed the lowest HbA1c levels. The maximum difference between medians of HbA1c in any two months of the study period equalled 0.85% (Aug-2008 vs Feb-2006). Linear, decreasing trend of HbA1c values over the study period was statistically significant (Beta=-0.39; $p=0.008$). Detrended residuals of HbA1c levels showed a sine-wave pattern of autocorrelations with a period of 12-14 months, suggestive of positive correlation between months one year apart and of negative correlations for intervals of 6-7 months (Figure).
Conclusion: 1) HbA1c levels in young T1DM patients are seasonally variable and the lowest levels may be found in late summer. 2) Seasonal change in HbA1c levels should be considered in clinical practice and in short-time (lasting several months) clinical trials or research schedules.

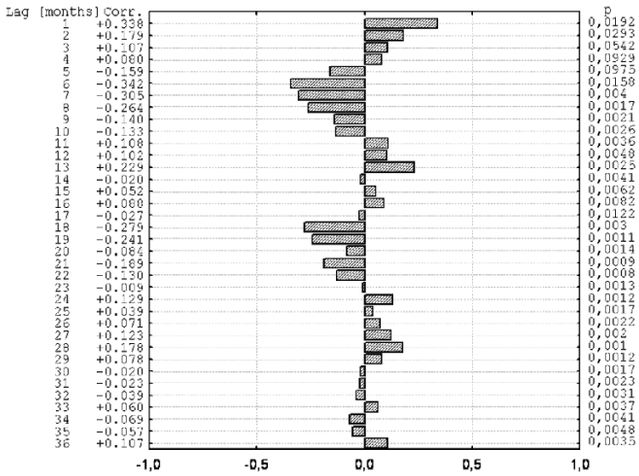

Figure – Autocorrelogram of HbA1c levels during the study period

Supported by: The Foundation for Polish Science, TEAM Programme 1.2 PO IG

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Epidemiology of type 1 diabetes mellitus in children in Uzbekistan

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Background and aims: Assessment and monitoring of basic epidemiological parameters of type 1 DM among children in the course of the 2000-2007 National Register.
Materials and methods: Epidemiological data was studied on the basis of annual reports from 13 regional endocrinological dispensaries and Tashkent endocrinological dispensary as well as on the basis of register cards filled up by local endocrinologists-pediatricians.
Results: In Uzbekistan within the period from 2000 to 2007 type 1 DM prevalence increased from 7.5 to 11.0 per 100,000 of pediatric population. The highest prevalence was observed in Tashkent (17.7), in Bukhara region (16.6) and in Tashkent region (14.5), the lowest one in Kashkadarya region (7.1) and in Surkhandarya region (7.7). Pediatric incidence in 2007 as compared with 2000 reduced from 2.7 to 2.1 per 100,000 of pediatric population. Analysis of pediatric incidence in 2007 revealed the highest one in Tashkent (4.6) and in Syrdarya region (2.7), the lowest being found in Navoi region (0.9). As to age distribution children aged from 10 to 14 comprised the largest group (66.6%),

the smallest including children from 0 to 4 years (5.0%), 28.3% accounting for patients aged from 5 to 9. As to the disease duration children with type 1 DM duration less than 5 years comprised the largest group (70.9%), in the smallest one (2.1%) including patients with 10-year DM duration. As a whole, in Uzbekistan within the period of the Register fulfilment mortality level reduced from 0.1 to 0.03 per 100, 000 of pediatric population. In 2007 mortality cases were registered in Kashkadarya region (0.1), Navoi region (0.4) and Samarkand region (0.09 per 100, 000 of pediatric population. As a whole in Uzbekistan mortality reduction in children with type 1 DM within the period from 1998 to 2007 was 99%.
Conclusion: Within the period of the National Register reduction in mortality paralleling alterations in structure of death cause and increase of survival can be noted to suggest perfect choice of strategy and tactics of the Register fulfillment.

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The impact of intrauterine hyperglycaemia on glucose metabolism in the offspring five years after delivery

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Background and aims: Diabetes in pregnancy is associated with a higher risk of obesity and abnormal glucose homeostasis for the offspring in later life. The aim of the study is to assess features of glucose metabolism and insulin secretion as well as the prevalence of obesity in young children of diabetic mothers.
Materials and methods: In a prospective cross-sectional analysis data on anthropometric and metabolic parameters of 58 children (f:29; m:29) aged 4-9 years were collected. 13 mothers of these children had at the time of pregnancy a normal glucose tolerance (NGT) and served as a control group whereas 31 mothers were affected by gestational diabetes (GDM) and 14 women by Type 1 diabetes (T1D). Differences in BMI-SDS (Standard Deviation Score) were suggested as the primary outcome of this study. Further, a 2h-OGTT was performed in all children and circulating levels of adiponectin and leptin were measured.
Results: Children of mothers with T1D (-0.83±0.94) showed significantly higher BMI-SDS as compared to GDM (-0.11±0.94; p=0.003) and NGT (-0.20±0.88; p=0.021) exposed children. There were no differences between the NGT and GDM subgroups (p=0.77). However, the glucose profile during the 2h-oGTT was comparable (G0:p=0.51; G60:p=0.26; G120:p=0.47) and also indices of insulin resistance were not different (HOMA:p=0.64; Quicki: p=0.64). Regarding adipokine levels, we found no differences for Leptin (p=0.33) but when comparing adiponectin levels of GDM and T1D groups we found higher levels for T1D children (p= 0.047).
Conclusion: Children of women with T1D in pregnancy were more obese than children of mothers with NGT and GDM despite comparable plasma leptin concentrations and similar degree of insulin sensitivity at the age of five years. Further longitudinal studies are needed to detect as early as possible those children at highest risk in follow-up.

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Plasma vitamin D and preservation of C-peptide in youth with recently diagnosed autoimmune positive type 1 diabetes: SEARCH Nutrition Ancillary Study

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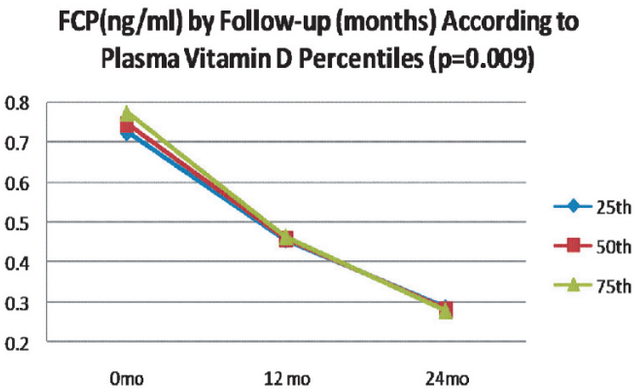
Background and aims: Preservation of insulin secretion following a diagnosis of diabetes predicts improved prognosis, yet little is known of the potential nutritional determinants of sustained beta cell function. We explored associations of plasma 25-hydroxyvitamin D concentration with baseline and short-term (~ 24 months) change in fasting c-peptide (FCP) within the SEARCH for Diabetes in Youth cohort diagnosed between 2002 and 2005.
Materials and methods: Included were 1224 youth (mean age at diagnosis, 9.8 yr) with at least one diabetes autoantibody positive (GAD65 or IA-2) and a clinical diagnosis of type 1 diabetes. About 77% were non-Hispanic white and gender was about equally distributed (52% male). Mean diabetes duration at baseline was 10 months. Mixed models regression analyses accounting for repeated measures were fit adjusting for onset age, sex, race/ethnicity, clinical center, baseline waist circumference, A1c, insulin regimen, HLA risk group and change in an index of insulin resistance. Vitamin D was analyzed as a continuous measure with values at the 25th, 50th, and 75th percentiles used for illustration.
Results: From the fully adjusted model, higher baseline vitamin D was associated with higher FCP at baseline (p=0.04) but unexpectedly was associated with more rapid decline in FCP (see figure; fully adjusted p-value=0.009).
Conclusion: Higher plasma vitamin D may provide some protection of the beta cell near the time of T1D diagnosis, although this protective effect appears to be of limited durability. The mechanisms for a potential protective effect of vitamin D on beta cell function near the diagnosis of diabetes, and whether such an effect would hold clinical significance, remains to be determined.



Supported by: NIH/NIDDK

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The benefits of continuous subcutaneous insulin infusion in children with type 1 diabetes mellitus started at diabetes recognition. A 7 year follow-up

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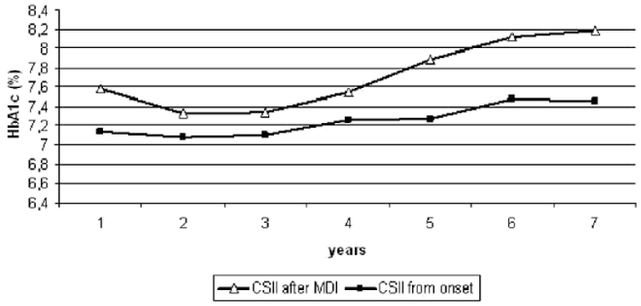
Background and aims: Intensive management, achieved by multiple daily insulin injections (MDI) or continuous subcutaneous insulin infusion (CSII), improves long-term outcomes in patients with Type 1 diabetes (T1DM). CSII and MDI are both effective in achieving near-euglycaemia, but CSII offers the opportunity to change the basal infusion rate according to the needs of patients and allows precise insulin dosing to cover a different kind of meals. In pediatric patients, CSII has been demonstrated to achieve glucose target and reduce frequency of severe hypoglycemia, without sacrifices in safety, quality of life, or excessive weight gain. In long-term follow up patients treated at T1DM onset with CSII therapy may achieve better metabolic control than patients switched from injections to CSII. The aim of this study was to compare efficacy of CSII at diabetes recognition with CSII after MDI in children with T1DM. The primary end-point was glycated haemoglobin.
Materials and methods: 169 children with T1DM treated with CSII for at least 5 years were divided into two groups depending on time of starting CSII therapy. I group: 97 children (girls) with mean age 6.7±2.3 years switched from MDI to CSII, the mean time with MDI therapy was 2.2±1.6 years; II group: 72 children (girls) with mean age 6.2±2.1 years started with CSII within 3 month after diabetes onset. Data was collected every three months: HbA1c, BMI, diabetic ketoacidosis (DKA) and severe hypoglycaemia (SH), total daily insulin dose was downloaded from pump memory.
Results: During the 7-year follow-up better metabolic control was achieved in the group II, the differences between group I and group II from the 2nd to the 4th year were not quite significant (2nd yr:7.33 vs. 7.08; 3rd yr: 7.33 vs. 7.09; 4th yr: 7.54 vs. 7.26%) , and statistically significant: in the 1st year (7.58 vs. 7.13%) and from the 5th to the 7th year (5th yr: 7.89 vs. 7.27; 6th yr: 8.11 vs. 7.48; 7th yr: 8.18 vs. 7.45%) p<0.05 . In the group II HbA1c was <7.5%

and in the group I increased to 8.2% at the time of follow-up. TDD increased from 0.4U/kg/d to 0.7U/kg/d p<0.0001. The insulin daily dose increased in the I group from 0,7 to 0.87 U/kg/die and in the II group from 0.48 to 0.87 U/kg/die, the significant differences between both groups were observed in the 1st and 2nd year. BMI increased in the I group from 16.3 after the 1st year to 19.8 kg/m2 after the 7th year and in the group II from 15.9 after the 1st year to 18.4 kg/m2 after the 7th year. BMI was significantly higher in the group I in the 6th and 7th year of the follow-up. There was no significant difference between both groups in the number of episodes of DKA and SH.
Conclusion: In longitudinal observation children started with CSII at diabetes recognition achieved better metabolic control than children initiated with CSII after MDI. The long- term benefits of CSII therapy started at T1DM onset should be taken under consideration before making a decision on CSII initiation.



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Long-term efficacy of insulin pump therapy in young children with diabetes
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Background and aims: To compare effectiveness of continuous subcutaneous insulin infusion (CSII) to multiple daily injections with rapid-acting insulin analogues and NPH insulin (MDIs) in preschoolers after more than 1 year of treatment.
Materials and methods: We evaluated 25 preschool patients (11 males, 14 females) with a history of type 1 diabetes of at least one year duration (14 CSII, 11 MDIs). Outcomes included measures of: glycated haemoglobin (HbA1c), mean blood glucose (BG) and standard deviation (SD), percent of BG values above and below target regarding the last 3 months of follow up, and the average daily risk range (ADRR) regarding the last month of follow up. Unpaired nonparametric two-tailed Mann-Whitney tests were used to analyze data from the two groups.
Results: The 25 subjects' ages ranged from 2 to 6 years (mean age CSII 4,6 yrs; mean age MDII 4.6 yrs), duration of diabetes ranged from 1 to 4.9 years (mean disease duration CSII: 2.9; mean disease duration MDIs: 2.3), duration of CSII ranged from 1 to 3.9 years (mean CSII duration 2.45 yrs). Comparison of overall metabolic control showed no statistically significant differences between the two groups when considering HbA1c (CSII 7.09%; MDIs 7.38%), mean BG (CSII 161.8 mg/dL; MDIs 168 mg/dL) and SD (CSII 82.1 mg/dL; MDIs 85.2 mg/dL), and percent of BG values below target (CSII 12,7%; MDIs 15.2%). However a statistically significant difference was found when comparing percent of BG values above target (CSII 35.1%; MDIs 47.2%; p<0.05). Furthermore, the MDIs group showed ADRR values consistent with moderate risk of BG excursions, while the CSII group showed an ADRR value consistent with high risk. However, no statistically significant differences were found regarding ADRR values of the two groups (CSII 44.9; MDIs 35.6).
Conclusion: Although CSII can be a safe and effective method to deliver insulin in young children, long-term pump therapy in our young patients was not associated with significant differences in glycemic control as compared with intensive injection therapy, confirming short-term results from past studies. Our study also showed that both CSII and MDII may allow the achievement of target glycemic control (HbA1c < 7.5 for all age-groups according to ISPAD 2009 guidelines), underlining that optimal control may be obtained independently of the means of insulin administration. Furthermore, evaluation of ADRR showed a greater risk for glycemic excursions in the CSII group, although comparison of ADRR values between the two groups showed no statistically significant difference. Consequently, rationale for initiating CSII in this age group should be primarily based on patient/parent selection and lifestyle preference. Nonetheless, further studies involving sensor augmented CSII in this age group are warranted in order to evaluate potential benefits on diabetic complications, thus going beyond lifestyle improvements which currently appear to be the only achievement in preschoolers.

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Intervention with metformin in childhood diabetes may slow decline of C peptide - the accelerator hypothesis
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Background and aims: Diabetes results from beta cell insufficiency. The accelerator hypothesis proposes that type 1 and type 2 diabetes are not different disorders, but poles of a single spectrum. Variable interaction between insulin resistance and the immune response to it determines the tempo of beta cell loss, and with it age at onset and incidence. The hypothesis is sufficiently well developed to consider use of metformin in children with new onset of type 1 diabetes. The aim of the study was to investigate the impact of metformin on the progression of childhood diabetes.
Materials and methods: Twenty-one children, mean age 10,9± 2,8y with recently diagnosed and insulin-treated type 1 diabetes were studied. Metformin was added after two weeks as insulin sensitizer and apoptosis reducing agent. Inclusion criteria were basal C peptide over 0.2 nmol/l and preserved pulsatility of insulin secretion. Twenty-six children and adolescents on insulin monotherapy acted as controls.
Results: Insulin was gradually reduced according to daily glycemic profiles and the metformin-treated children entered remission faster. Six of them achieved complete remission for longer than 12 months. Mean C-peptide level after 18 months was 0.57 nmol/l in metformin group versus 0.20 in the group on insulin alone (p<0.05). HbA1C tended to be lower in the metformin group; (7.7 % compared with 9.0 % , NS) .
Conclusion: Our first experiences with combined treatment (insulin and metformin) are favourable, suggesting that metformin use in childhood diabetes may reverse C peptide decline. Further studies are necessary.

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Relationship between depressive symptoms and quality of life and metabolic control in children and adolescents with diabetes type 1
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[1]Clinical Pediatric Hospital, [2]The Department of Pediatrics, Clinical Pediatric Hospital, [3]The Department of Pediatrics, Medical University of Warsaw, Poland.

Background and aims: As many studies show depression is a factor involved in pathogenesis of diabetes and a major complication affecting metabolic control in patients with diabetes. There is a higher incidence of diabetes among patients with depression and patients with diabetes and depressive symptoms achieve worse metabolic control. These studies are usually conducted on adults with diabetes type 2. It is still little known about children and adolescents with diabetes type 1. The aim of this study was to assess the prevalence of depression and examine its effect on metabolic control and quality of life in children and adolescents with diabetes type 1.
Materials and methods: 214 children so far took part in this study: 107 girls and 107 boys. Mean age: 13.1 <7-17, SD 2.7>. Mean diabetes duration: 5.2 (SD 2.7). During the routine visit in the outpatient clinic all children and adolescents with diabetes type 1 age 7 and above were asked to fill in Children's Depression Inventory (Polish version), a self-report questionnaire consisting of 27 items. Patients from age 11 and above were asked to answer questions in Quality of Life Questionnaire, a 58 item questionnaire based on the DCCT Diabetes Quality of Life Measure. At the same time other data was collected: sex, age, diabetes duration, HbA1c, BMI, daily insulin dose.
Results: 35 participants (16.35%) scored ≥ 13, indicating elevated depressive symptoms. In the group with scores below 13 (179) there were 76 participants (42.45%) who scored above average in one of subscales. There was an extremely significant correlation between scores on the CDI and quality of life (r=0.6510, p<0.0001). A very significant correlation was found between scores on the CDI and HbA1c (r=0.2088, p=0.005) and age of participants (r=0.2000, p=0.005). There was also a significant correlation between scores on the CDI and diabetes duration (r=0.1576, p=0.023), BMI (r=0.1356, p=0.049) and insulin daily dose (r=0.1785, p=0.011). There was no difference between girls and boys on the CDI scores.

Conclusion: 16.35% participants show elevated depressive symptoms as assessed with Children's Depression Inventory. 42.45% participants without depressive symptoms score above the average on one of the CDI subscales. Children and adolescents with higher scores on the CDI have worse quality of life and achieve worse metabolic control. The longer diabetes duration and the older participant the more likely he presents depressive symptoms. It is necessary to pay attention to emotional wellbeing of children and adolescents with diabetes type 1, especially the older ones who are longer ill. It is necessary to develop an intervention program aimed at prevention of emotional problems in youths with diabetes.

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Longitudinal associations between depressive symptoms and glycaemic control among adolescents and young adults with type 1 or type 2 diabetes

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[1]Kaiser Permanente Southern California, Pasadena, [2]Cincinnati Children's Hospital, Cincinnati, [3]Wake Forest Univ., Winston-Salem, [4]Univ. of Washington, Seattle, [5]Univ. of Colorado Denver, [6]Centers for Disease Control and Prevention, [7]Univ. of South Carolina, Columbia, USA.

Background and aims: Depression is associated with poor glycemic control in youth with diabetes (DM). We assessed the association between depression scores and change in depression score over time with change in A1c among youth in the SEARCH for Diabetes in Youth study.
Materials and methods: 861 youth (age 15.3 ± 2.5 years; 78% type 1 DM; 50% female; 81% non-Hispanic White) had a baseline visit (time1) within ≈ 1 year of DM diagnosis and completed a follow-up visit (time2). At each visit, symptoms of depression were evaluated with the Center for Epidemiologic Studies-Depression (CES-D) scale, blood was drawn for A1c analysis, and DM duration was assessed. CES-D score was categorized to indicate minimal (0-15), moderate (16-23) and frequent (24-60) symptoms of depression, and also used as a continuous measure in regression analyses. A linear regression model was fit to examine the effects of CES-D score at time1 and the change in CES-D score from time1 to time2 (predictors) on the change in A1c from time1 to time2 (outcome) adjusting for age, gender, race/ethnicity, DM type, DM duration, BMI-Z score, and presence of co-morbidities, all measured at time1, as well as time between visits.
Results: Youth had DM for 26.8±9.1 months at their time2 visit which was, on average, 17.2±6.0 months after their time1 visit. At time1, 80%, 13%, and 8% had minimal, moderate, and frequent symptoms of depression, respectively, while a similar distribution observed at time2. CES-D category at time1 and time2 were significantly associated ($p<0.001$); continuous scores on the CES-D were also correlated ($r=0.56$, $p<0.0001$). Of youth with minimal symptoms at time1, 87% remained minimal at time2 while 8% were moderate and 5% had frequent symptoms of depression. Of youth with moderate symptoms at time1, 56% improved while 17% moved to the frequent category at time2. Of those with frequent symptoms at time1, 34% stayed the same, while 29% were in the moderate and 37% in the minimal symptom categories. In the multiple linear regression analyses, there was a positive association between both the CES-D score at time1 and change in CES-D from time1 to time2 and change in A1c. Larger changes in CES-D and higher CES-D scores at time1 were both associated with greater increases in A1c. Race/ethnicity, gender, DM duration and BMI-Z were also significant in the final model while DM type was not.
Conclusion: Most youth with minimal symptoms at time1 remained in that category at time2 while about 2/3 of youth with frequent symptoms experienced some improvement. Youth who had more frequent symptoms of depression as well as a greater increase in symptoms tended to have worsening glycemic control at follow-up while improvement in depressive symptoms was associated with improved glycemic control. Screening for and treatment of depressive symptoms in adolescents and young adults with DM may help improve glycemic control in this population.
Supported by: U.S. CDC & NIH

PS 86 Nutrition and diet

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Dose-dependent effects of protein 'preloads' on gastrointestinal hormones, glycaemia, and energy intake in type 2 diabetes

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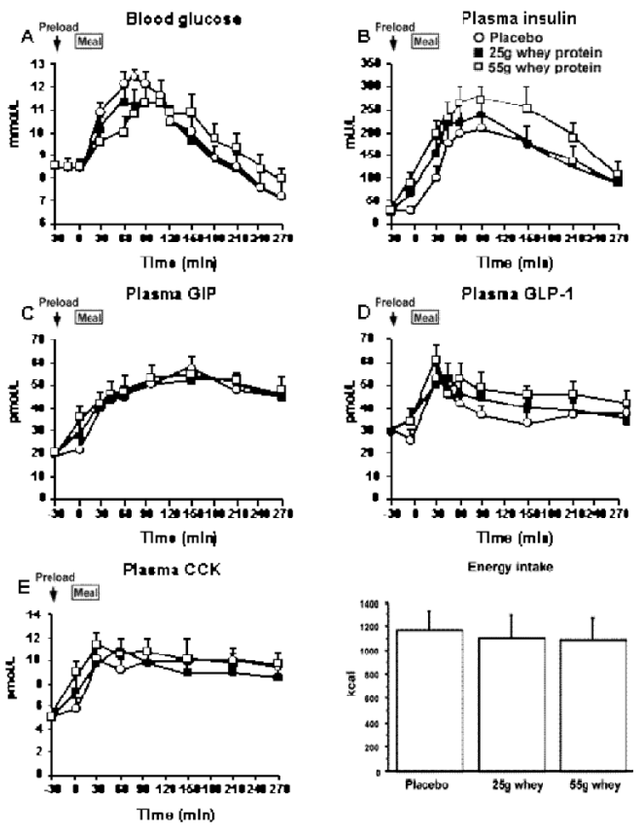
Background and aims: Whey protein 'preloads' can stimulate glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), insulin and cholecystokinin (CCK) release, slow gastric emptying of a subsequent meal, and improve postprandial glycaemia. We aimed to determine the effects of different doses of whey, when ingested before a buffet-style meal, on gastrointestinal hormones, glycaemia, and energy intake in type 2 diabetes.
Materials and methods: Nine patients with diet-controlled type 2 diabetes (glycated haemoglobin 6.7 ± 0.3 %) were studied on 3 separate days in randomized order. Subjects consumed a chocolate-flavoured liquid 'preload' (100 ml water mixed with either a flavoured 'placebo' (8 kcal), or with 25 g (89 kcal) or 55 g (195 kcal) flavoured whey protein), 30 minutes before an ad libitum meal (T = 0 to 30 min). Blood was sampled frequently for hormone measurements.
Results: Data are shown as mean ± standard error. Both whey preloads stimulated GLP-1, GIP, insulin and CCK before the meal ($P < 0.05$); the stimulation of insulin and CCK were greater ($P < 0.05$) with 55 g whey. The incremental area under the curve (iAUC) for GLP-1 was greater after 55 g whey than the other days, while iAUC for insulin was greater after 25 g and 55 g whey than placebo. The peak postprandial blood glucose was slightly lower with 25 g and 55 g whey than placebo ($P < 0.05$). Both whey preloads increased postprandial fullness slightly ($P < 0.05$), but neither affected energy intake.
Conclusion: Acute administration of a whey protein 'preload' in patients with diet-controlled type 2 diabetes dose-dependently stimulated GIP, GLP-1, insulin and CCK, and reduced postprandial glycaemia, as well as increasing fullness, but had no significant effect on energy intake.



Supported by: NHMRC

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Sustained effects of a protein 'preload' on gastric emptying and glycaemia in type 2 diabetes over 4 weeks

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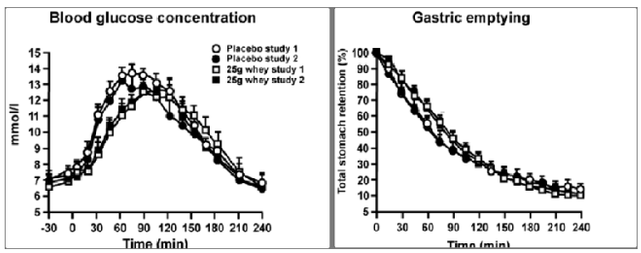
Background and aims: Whey protein 'preloads' acutely reduce glycaemia after a subsequent meal in type 2 diabetes, associated with slowing of gastric emptying and stimulation of incretin and insulin release. The aim of the current study was to evaluate whether the effects of protein preloads on gastric emptying and glycaemia are sustained with 'chronic' (4 weeks) administration.
Materials and methods: Seven patients with uncomplicated type 2 diabetes treated by diet alone (glycated haemoglobin 5.9 ± 0.2 %) participated in the study. Each consumed a chocolate-flavoured 'preload' (containing either 25 g whey or placebo), 30 min before each of the three main meals for 4 weeks, followed by a 'washout' period of 2 weeks, and then the alternative preload for 4 weeks, in a randomized crossover design. Gastric emptying of (scintigraphy), and the glycaemic response to, a standard potato meal consumed 30 min after the preload, were measured at the beginning and end of each 4 week period, as was serum fructosamine.
Results: Data are shown as mean ± standard error. Whey slowed gastric emptying and reduced postprandial blood glucose compared to placebo, both at baseline and after 4 weeks exposure to whey (repeated measures ANOVA, $P < 0.05$ for all comparisons), without any difference between baseline and 4 week values. Fructosamine was non-significantly lower after 4 weeks whey than placebo (253 ± 15 vs 279 ± 10 μmol/L, $P = 0.15$).
Conclusion: Administration of a whey preload for 4 weeks results in sustained slowing of gastric emptying and reduction in postprandial glycaemia in type 2 diabetes. A larger trial of longer duration is indicated to determine whether this strategy can improve glycaemic control.



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Thorough chewing stimulates postprandial increases of plasma GLP-1 and peptide YY in normal subjects

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Background and aims: Glucagon like peptide (GLP)-1 and peptide YY (PYY) are secreted from intestinal L cells, and plasma levels of both hormones rise after a meal. GLP-1 stimulates glucose-dependent insulin secretion. PYY decreases appetite and reduces food intake by acting on receptors in the hypothalamus. GLP-1 also reduces food intake. Therefore, GLP-1 and PYY seem important to control plasma glucose and triglyceride (TG) levels and body weight. On the other hand, it has been conventionally thought that "chewing well" i.e. "thorough chewing" is good for health. Much remains unknown on the relationship between thorough chewing and GLP-1 and PYY which are secreted from intestine after a meal. The aim of this study is to investigate effects of thorough chewing on postprandial levels of plasma GLP-1 and PYY in normal subjects.
Materials and Methods: Twenty two normal subjects were recruited. They were not obese and not diabetic. Plasma mean fasting glucose was 96 mg/dl. Mean age was 37 years and mean BMI was 23.1. The subjects were given the test meal early in the morning after 12h fasting. They ate it for 20 minutes and chewed each mouthful 5 times (5 times chewing). On the other day the subjects ate it for 20 minutes and chewed each mouthful 30 times (30 times chewing, i.e. thorough chewing). Plasma GLP-1 and PYY were measured before and 1 h after ingestion of the test meal. Plasma glucose and insulin were measured before and 1 h after ingestion of the test meal. Plasma TG was measured before and 2 h after ingestion of the test meal. The test meal consisted of bread, margarine, a boiled egg, steamed vegetables, a banana, and milk. Total calories were 630 kcal, with 16% protein, 32% fat, and 52% carbohydrate. Plasma PYY was measured as PYY(3-36) using an enzyme immunoassay kit. Plasma GLP-1 was measured as GLP-1(7-36) using ELISA kit.
Results: Plasma mean PYY levels with 5 times chewing tended to increase from 41.0 pg/ml (before a meal) to 46.1 pg/ml (after a meal). Plasma PYY levels with 30 times chewing significantly increased from 41.7 pg/ml (before a meal) to 65.4 pg/ml (after a meal). Postprandial PYY level with 30 times chewing was significantly higher than with 5 times chewing. Plasma mean GLP-1 levels with 5 times chewing significantly increased from 4.8 pmol/l (before a meal) to 18.9 pmol/l (after a meal). Plasma GLP-1 levels with 30 times chewing significantly increased from 5.0 pmol/l (before a meal) to 25.1 pmol/l (after a meal). Postprandial GLP-1 level with 30 times chewing was significantly higher than with 5 times chewing. Plasma mean TG level with 5 times chewing significantly increased from 107 mg/dl (before a meal) to 170 mg/dl (after a meal). Plasma TG level with 30 times chewing significantly increased from 114 mg/dl (before a meal) to 147 mg/dl (after a meal). Postprandial TG level with 30 times chewing was significantly lower than with 5 times chewing. There was no significant difference in increases of plasma glucose and insulin after a meal between 5 times and 30 times chewing in normal subjects.
Conclusions: This is the first report that thorough chewing stimulates postprandial increases of plasma GLP-1 and PYY in normal subjects. In addition, thorough chewing suppresses postprandial increase of plasma TG level. Thorough chewing may be clinically effective in normal subjects.

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Carbohydrate substitution for protein, fat, and their subtypes and risk of type 2 diabetes in men

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Background and aims: Interest in optimal macronutrient proportions to avoid type 2 diabetes has grown recently. We examined the associations of intakes of carbohydrates, protein, fat, and their subtypes (low, medium, and high GI carbohydrates; protein from meat, milk products, and plant sources; and fatty acids) with diabetes risk.
Materials and methods: The cohort comprised 25 943 Finnish male smokers aged 50-69 years. Diet was assessed at baseline with a validated diet history questionnaire. During a 12-year follow-up, 1 098 incident diabetes cases were identified from a national register. Cox proportional hazard modeling was used to estimate the risk for diabetes and multivariate nutrient density models to examine the effects of substitutions of different macronutrients.
Results: The substitution of carbohydrates for protein was inversely associated with diabetes risk: change in the multivariate relative risk when carbohydrates replaced two percent of energy of protein was 0.85 (95% CI: 0.80, 0.90). The substitutions of carbohydrates for protein subtypes, protein from meat, milk products and plant origin, were each inversely associated with diabetes risk, but plant protein intake itself showed no association with diabetes risk. The substitution of carbohydrates for total fat was also inversely associated with diabetes risk, but not significantly for all the fatty acids.
Conclusion: Greater carbohydrate intake at the expense of protein, especially from meat or milk products, was associated with decreased diabetes risk. Carbohydrate intake at the expense of total fat intake was associated with decreased diabetes risk, but the beneficial association may depend on which fatty acids are substituted.
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Effect of changes in the intake of specific food groups on weight loss; a two year dietary intervention trial

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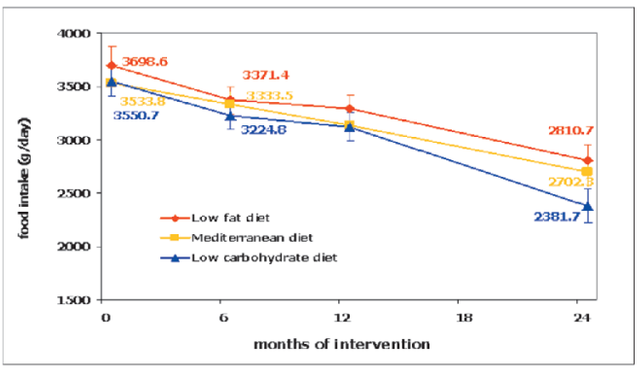
Background and aims: Adherence to distinct dietary strategies is associated with changes in specific food-groups consumption. We aimed to address the effect of changes in the intake of specific *weight* of food-groups on weight-loss in a 2-year low-fat, Mediterranean and low-carbohydrate dietary intervention trial.
Materials and methods: Electronic-food-frequency questionnaires were used to assess changes in the intake of 11-food-groups (beverages, vegetables, fruits, dairy products, meat, breads/cereals/pasta/potatoes, sweets/cakes, legumes, fish, fats/oils, and eggs) among patients with type 2 diabetes (n=45) and non-diabetics (n=277) moderately obese participants (BMI=31kg/m^2; age=52years; 86%men).
Results: Mean weight-losses at 6-months were -4.6kg, -4.7kg and -6.4kg for the low-fat, Mediterranean and low-carbohydrate groups, respectively ($p<0.026$ between groups). Reduction in total weight of food consumption, however, was similar across diet-groups (Figure 1): from 3,593 g/day, the participants reduced ($p<0.005$) their food intake by -284g/day at 6-months and by -963g/day at 24-months. In multivariate regression models, adjusted for age, sex, baseline body-weight and simultaneous *changes* of 11-food-groups weight intake (g/day), independent dietary predictors of 6-month weight-loss (rapid weight-loss-phase) were: decreased consumption of sweets and cakes ($\beta=0.493;p=0.008$) in the low-fat, increased intake of crude legumes ($\beta=-0.196;p=0.061$) in the Mediterranean, and increased vegetables intake ($\beta=-0.249;p=0.018$) in the low-carbohydrate diet-group. In the entire study population, in models further adjusted for diet-type, the leading predictors for weight loss after 6-months were increased vegetables ($\beta=-0.116;p=0.045$) and decreased sweets and cakes intake ($\beta=0.162;p=0.010$). Predictors for 2-year successful weight loss in the entire group were: increased vegetables ($\beta=-0.192;p=0.007$) and meat ($\beta=-0.146;p=0.026$) and decreased eggs ($\beta=0.187;p=0.003$), processed legumes ($\beta=0.195;p=0.002$), and beverages intake ($\beta=0.135;p=0.032$). Analyses were similar when stratifying by diabetes status.
Conclusion: Weight loss may be achieved by a variety of changes in specific food-groups consumption within different diet strategies, while in overall, the leading universal predictors for weight loss are increased vegetables and decreased sweets and cakes intake.

Figure 1: Total weight of food intake (g/day) at baseline, 6 and 24 months, across dietary intervention groups



- tested with ANOVA for between groups and with paired Student t-test for within group comparisons. N=321.
- All diet groups reduced significantly their food intake from baseline to 6 and 24 months, (within groups $p<0.005$).
- No significant differences were observed between the diet groups. Vertical bars indicate standard errors

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Special dietary regimen and nutrient intake of patients with type 1 diabetes

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Background and aims: Patients with type 1 diabetes are instructed to eat a healthy, balanced diet with the aim to optimize metabolic control. Type 1 diabetes is, however, associated with a number of conditions that may require dietary modifications. We aimed to evaluate the frequency of adhering to special dietary regimen and to study whether such adherence would rise any concern in the sufficiency of dietary intake.
Materials and methods: Cross-sectional data were collected from 810 participants [40% men, mean age 47 (range 18-84) years, diabetes duration 32 (1-68) years] in the Finnish Diabetic Nephropathy Study. Data on energy and nutrient intake were collected with a three-day food record (two weekdays and a weekend day) that was completed twice with a 2-3 month interval. Self-report questionnaire was applied to assess adherence to a lactose free, protein restriction, gluten free, or vegetarian diet. Whether diet was introduced based on a recommendation from a health care professional or was self-initiated, was also enquired.
Results: A total of 225 (28%) patients reported adhering to a special diet. Adherence to a lactose free diet was the most frequent (15%), followed by protein restriction (7%), vegetarian (6%) and gluten free diet (4%). Vegetarian (93%) and lactose free (63%) diets were most frequently self-initiated, while gluten free and protein restriction diets were primarily initiated based on a diagnosis (88% and 85%, respectively). Frequencies of reaching the recommendations for carbohydrate intake (45-60 E%) ranged from 50% (gluten free) to 63% (vegetarian). Those not reaching the recommendations mainly consumed less carbohydrates than recommended. A total of 24% (protein restriction) to 37% (vegetarian) of the patients exceeded the recommendations for sucrose intake (<10 E%). Recommendations for protein intake (10-20 E%) were frequently met in all four groups (86 to 92%). At least 50% of patients in all groups met the recommendations for total fat (25-35 E%), monounsaturated fatty acids (10-20 E%), and polyunsaturated fatty acids (5-10 E%). However, a substantial proportion of patients exceeded the recommended levels for total fat (28 to 46%) and saturated fatty acid (<10 E%) intakes (61 to 84%). Moreover, the proportion of patients achieving the recommendations for fibre (0 to 3%) and salt intake (30 to 50%) were low in all four groups. Mean intakes of vitamin A and D per 1000 kcal were below recommendations in all special diet groups. The mean intake of folic acid was below recommendations among all but those adhering to a vegetarian diet. Moreover, those on a gluten free diet did not, in average, meet the recommended level for iron intake.
Conclusion: Special diets were found to be common among patients with type 1 diabetes. Dietary intake of those following a special dietary regimen did not, for many parts, meet the recommendations. Particular attention should be paid to fibre, saturated fatty acid, salt, vitamin A and vitamin D intakes. Moreover, those adhering to gluten free diets are encouraged to address their iron intake.
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Glycaemic responses of RP-13 (Bangladeshi Origin) and Jasmine (Thailand) rice in type 2 diabetic subjects

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Background and aims: In the context of the importance of Glycemic Index (GI) and Glycemic Load (GL, an applied tool calculated from GI and serving size) in determining the health of carbohydrate rich foods. The present study was carried out to determine these parameters for Jesmine Rice (Thailand origin) and RP-13 rice (produced in Bangladesh by ISIS Food Products, Denmark), Insulinemic response and NEFA response, as important covariables, were also determined.
Materials and methods: Eighteen diabetic subjects (male 12, female 6), under a cross-over design, consumed equi-carbohydrate amount of the test

foods and two times glucose (as reference food), with a run in period of 7 days between the consecutive items. The (mean±SD) age (years) of the patients was 41± 6, waist hip ratio 0.93 ± 0.04, body mass index (BMI) 25 ± 3 and they had basal serum HbA_1c value of 6.56 ± 0.83 %. The test meals contained 50 g of total carbohydrate and were given to the participants for ingestion within 10 minutes with 200 ml water. Serum levels of glucose were estimated at 0, 30, 60, 90, 120, 150 and 180 minutes respectively. NEFA, TG and Insulin levels were measured at 0 and 180 minutes only. Serum glucose and TG were measured by glucose-oxidase method, serum Insulin by chemiluminescent ELISA, NEFA by colorimetric method and HbA_1c was measured by HPLC method.

Results: RP-13 showed significantly lower serum glucose value than that of Glucose and Jasmine rice (Incremental area under the curve 289.4±111.3 in RP-13 vs. 591.4±198.8 in Glucose and 499.1±180.3 in Jasmine Rice; (*p<0.001*)). RP-13 had significantly lower GI value than that of Jasmine rice [(Mean±SD): 51.5 ± 8.3 in RP-13 vs. 86.4 ± 21 in Jasmine rice (*p<0.001*)]. The GL of RP-13 and Jasmine rice are 22 and 41 respectively. There were no significant difference between RP-13 and Jasmine Rice regarding serum Insulin, serum TG and serum NEFA responses.

Conclusion: As judged against the mean values of the international table (GI: High ≥ 70, Medium 56-69 and low ≤ 55; GL: High ≥ 20, Medium 11-19 and low ≤ 10) RP-13 is a low GI and Jasmine is a high GI rice. However, from the dietary practices in Bangladesh both the items may be used as high GL rice. The data emphasizes the necessity of constructing GI and GL tables based on studies from individual societies.

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Both higher serum vitamin D and dairy calcium intake are related to a greater 2-year diet induced weight loss, especially in diabetes

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Background: The role of serum 25-OH vitamin D levels and dairy calcium intake on weight loss is controversial.

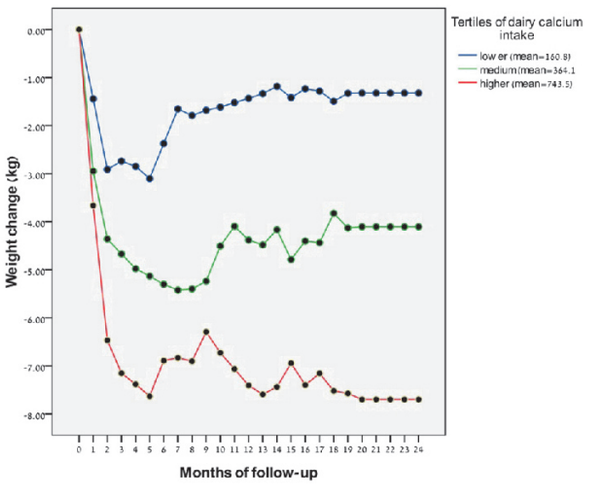
Objective: To address the association of dairy calcium intake and serum 25-OH vitamin D levels with long-term weight loss in persons with type 2 diabetes (T2D) and with persons with normal glucose tolerance (NGT).

Design: We analyzed the 2-year Dietary Intervention Randomized Controlled Trial (DIRECT)[NGT: n=277, T2D: n=45, mean body-mass-index (BMI)=31kg/m^2;mean age=52years]. A representative sample (n=126) was followed for 6-months for serum vitamin D changes. Dietary intake, including dairy products, was evaluated by a validated food frequency questionnaire (FFQ).

Results: Serum baseline 25-OH Vitamin D levels were significantly lower within the higher tertile of baseline BMI in both groups (entire group: 25.6ng/m, 24.1ng/ml and 22.9ng/ml; p for tend=0.02). Baseline levels of vitamin D and dairy calcium intake were not associated with subsequent weight loss. In repeated measures models, adjusted for age, sex, baseline BMI, total fat intake and diet group assignment, the six months tertiles of dairy calcium intake (mean for tertiles: T2D: 160.8mg/day, 364.1mg/day, 743.5mg/day, NGT: 153.3mg/day, 353.9mg/day, 638.2mg/day) and the six months tertiles of serum 25-OH vitamin D (T2D: 14.0ng/ml, 21.1ng/ml, 30.4ng/ml, NGT:13.8ng/ml, 20.8ng/ml, 31.0ng/ml) were associated with weight loss across the two-years of intervention. However, these associations were much stronger in persons with T2D. (-1.3kg; -4.1kg and -7.7kg; across tertiles of dairy calcium; p=0.018, and -0.8kg, -5.3kg and -8.7kg; across tertiles of serum 25-OH vitamin D; p=0.042).

Conclusion: Higher dairy calcium intake and increased serum vitamin D are related to greater dietary-intervention two-year weight loss, especially among persons with type 2 diabetes.

Figure 1: Adjusted* weight change among diabetics across 6 months tertiles of dairy calcium intake (reflecting the dairy intake in the previous 6 months)



* Adjusted means, repeated measure model, adjusted for age, sex, baseline BMI, total fat intake and assigned diet group. Mean levels of dairy calcium intake tertiles among T2D=160.8±62.0mg/day, 364.1±44.3mg/day, 743.5±202.7mg/day, respectively.

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Nutritional assessment in type 2 diabetic elderly patients admitted to an internal medicine ward

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Background: Type 2 Diabetes is more prevalent in elderly people (≥ 65 years) contributing to a major malnutrition risk in this age group. There are few data about nutritional state in elderly diabetic patients in Portugal.

Aim: The authors pretended to evaluate nutritional state in type 2 diabetic patients over 65 years admitted to the internal medicine ward of Hospital of Nossa Senhora da Assunção of ULS Guarda EPE.

Materials and methods: The authors evaluated consecutively 103 elderly diabetic patients from February to December 2009 and compared them to a group of 115 non-diabetic elderly patients. The clinical tool was the Mini Nutritional Assessment (MNA) who stratifies nutritional state from 0 to 30 in: malnutrition (<17), malnutrition risk (17,5-23,5) and normal (>24). Statistical analysis was performed by SPSS 13.0 for Windows, when appropriate.

Results: The 103 elderly diabetic patients (59,2% female and 40,8% male) had a mean age of 81,55 ± 7,08 years. There was no statistical significant difference from the 115 non-diabetic control group (59,1% female, 40,9% male with a mean age of 83,27 ± 7,02 anos, p > 0,05). Mean MNA score in Diabetic elderly patients was 13,64 ±4,11 which was significantly lower than in the control group (16,50±4,46, p < 0,001). In Diabetic patients, 13,6% had a normal score evaluation at screening and did not need to follow MNA evaluation, 66 % were classified with malnutrition , 19,4 % with malnutrition risk and 1 % were normal (values of 40,9%, 27,8%, 23,5% e 7,8% respectively for control group, p<0,001).

Conclusion: This study suggests that malnutrition is more prevalent in elderly people with type 2 diabetes admitted to a medical ward for various reasons, which implies that they must be identified for specific nutritional intervention. There will be necessarily more studies to evaluate the causes and the complex relationship between type 2 diabetes and malnutrition in elderly patients.

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N-3 fatty acids as phospholipids are superior over triacylglycerols in ameliorating hepatic steatosis in mice fed a high-fat diet

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Background and aims: n-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), could prevent development of obesity and insulin resistance. In this study, metabolic consequences of dietary n-3 PUFA supplemented to a high-fat diet either as phospholipids (PL) or triacylglycerols (TG) concentrates were studied in the obesity-prone C57BL/6J strain of mice.
Materials and methods: In the *Prevention* study, 3-mo-old male mice were fed *ad libitum* for 9 weeks either a corn oil-based high-fat diet (cHF; lipids ~35% wt/wt) or cHF-based experimental diets, matched for the total EPA and DHA content (3.15% wt/wt), in which part of dietary lipids (corn oil) was replaced by the EPA and DHA concentrates (EPAX a.s., Aalesund, Norway) in the form of either TG-concentrate (EPAX 1050 TG; 60% EPA+DHA wt/wt) or novel PL-concentrate (27% EPA+DHA wt/wt). In the *Reversal* study, obesity was induced by cHF feeding for 4 mo prior to dietary treatments by either TG- or PL-concentrates. Obese mice were then subjected to one of the following treatments: 1) cHF diet; 2) cHF and metformin (2 g/kg diet; cHF+M diet); 3) cHF+M and TG-concentrate (cHF+M+TG diet); 4) cHF+M and PL-concentrate (cHF+M+PL diet). Markers of glucose and lipid homeostasis, glucose tolerance (AUC), hepatic steatosis, adipocyte hypertrophy and adipose tissue inflammation were analyzed.
Results: In the *Prevention* study, the concentrates did not affect weight gain or adiposity, while reducing plasma NEFA (cHF, 0.44 ± 0.04 vs. TG-concentrate, 0.30 ± 0.02 vs. PL-concentrate, 0.27 ± 0.04 mmol/l; $p \leq 0.01$ cHF vs. either concentrate). PL-concentrate more effectively ($p<0.05$ vs. cHF) reduced plasma TG (cHF, 1.12 ± 0.13 vs. TG-concentrate, 0.91 ± 0.13 vs. PL-concentrate, 0.71 ± 0.13 mmol/l) and increased high-molecular weight adiponectin (cHF, 0.60 ± 0.11 vs. TG-concentrate, 0.88 ± 0.10 vs. PL-concentrate, 0.93 ± 0.09 A.U.). Only PL-concentrate ($p<0.01$) improved glucose tolerance during a 3-hr tolerance test (AUC: cHF, 2221 ± 78 vs. TG-concentrate, 2299 ± 84 vs. PL-concentrate, 1831 ± 84 mmol), and prevented hepatic lipid accumulation (cHF, 41 ± 7 vs. TG-concentrate, 36 ± 4 vs. PL-concentrate, 27 ± 4 mg/g tissue). In the *Reversal* study, both concentrates reduced abdominal fat depot, plasma TG, NEFA and cholesterol, and induced adiponectin. However, hepatic steatosis was more effectively reduced by PL-concentrate (cHF, 212 ± 28 vs. cHF+M, 160 ± 17 vs. cHF+M+TG, 83 ± 15 vs. cHF+M+PL, 41 ± 4 mg/g tissue; $p<0.001$ cHF+M vs. either treatment), and only PL-concentrate ($p<0.05$ vs. cHF+M) reduced adipocyte hypertrophy (cHF, 5532 ± 553 vs. cHF+M, 5961 ± 381 vs. cHF+M+TG, 5195 ± 349 vs. cHF+M+PL, 4433 ± 127 μm^2).
Conclusion: As compared to TG, dietary n-3 PUFA administered as PL exert a number of superior effects on obesity-associated metabolic disorders. The PL-concentrate was especially effective in reducing hepatic lipid accumulation and adipocyte hypertrophy associated with high-fat feeding. Thus, the use of n-3 PUFA as PL might be a preferred way of dietary supplementation to help prevent or even reverse hepatic steatosis associated with obesity and insulin resistance.
Supported by: IGA of the Ministry of Health of the Czech Republic

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The effects of omega-3 polyunsaturated fatty acids on cardiometabolic parameters and oxidative stress after 1-year administration in metabolic syndrome patients

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Background and aims: To observe if one year administration of a diet containing omega-3 PUFA supplements vs. baseline diet recommended to patients with metabolic syndrome has a significant impact on oxidative stress, atherosclerosis progression and metabolic parameters.
Materials and methods: A total of 270 patients with metabolic syndrome (MS) according to IDF criteria, aged 61±6.8 years, without clinical evidence of atherosclerosis were allocated to 2 groups, matched by sex, age and weight: group A (140 patients) - diet according to ESC/EASD recommendations and individual needs; group B (130 patients) - the same diet + capsules of fish oil (1,0 g eicosapentanoic acid, 1,0 g docosahexanoic acid and 0,1 g α-tocopherol acetate). Body fat mass (BFM) and body fat percent (%BF) were measured by bioimpedance analysis (BIA) using InBody 3.0 Analyzer. Fasting plasma glucose, HbA1c, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, plasma insulin, adiponectin and leptin were measured according to standard procedures. Insulin resistance was measured using HOMA-IR index. Oxidative stress was assessed using FormOx systems monitor on a blood drop. The progression of atherosclerosis was determined by measuring intima-media thickness (IMT) at commune carotid artery (ACC). The patients were evaluated at baseline, after 6 months and after 1 year.
Results: Baseline characteristics were similar between groups. After 6 months, omega-3 supplements determined a significant improvement of metabolic parameters, decrease of oxidative stress and a statistically significant increase in adiponectin levels (from 9.46 ± 2.76 to 10.86 ± 2.68). Mean BMI, mean %BF, mean BFM and mean waist-to-hip ratio (WHR) were significantly lower in group B vs. group A (BMI- 29.1 vs 31.12 kg/m^2; %BF - 27.48 vs 30.48; BFM - 26.78 vs 29.42 kg; WHR - 1.02 vs 1.07). BMI was statistically correlated with BFM ($p<0.0001$) and %BF ($p<0.0001$). Intima-media thickness (IMT) was significantly decreased in group B (IMT in left ACC - 0.610 ± 0.06 vs. 0.621 ± 0.071 mm p=0.002; IMT in right ACC - 0.593 ± 0.074 vs. 0. 612 ± 0.068). Considering the results at 6 months comparing with those at 1 year, all the parameters considered in the study were significantly improved (Table 1). IMT was correlated with %BF ($p<0.0001$), WHR (p=0.002), leptin values ($p<0.001$), adiponectin values ($p<0.001$), leptin/adiponectin ratio ($p<0.001$) and oxidative stress ($p<0001$). The decrease of oxidative stress was correlated with increased HDL-cholesterol levels ($p<0.05$), %BF ($p<0.0001$) and WHR ($p<0.001$).
Conclusion: Omega-3 PUFA enriched diets bring metabolic parameters closer to target values, thus lowering cardiovascular risk of MS patients. Also, oxidative stress is decreased, underlying the role of omega-3 in the delay of endothelial cells damage.

Table 1

Parameters	Group B - at 6 months	Group B - at 1 year	P value
Total cholesterol (mg/dl)	198 ± 18.9	186 ± 16.5	P<0.001
HDL-cholesterol (mg/dl)	55 ± 12	58 ± 9	P<0.0001
Triglycerides (mg/dl)	132 ± 58	120 ± 43	P=0.012
Fasting Plasma Glucose (mg/dl)	110 ± 14	107 ± 9	P<0.05
FormOx (Fort Units)	268 ± 76	258 ± 81	P<0.001
IMT - right ACC	0.610 ± 0.06	0.598 ± 0.082	P<0.001
IMT - left ACC	0.593 ± 0.074	0.589 ±0. 063	P<0.05
%BF	27.48 ± 2.8	25.92 ± 1.6	P=0.016

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Metabolic inflexibility to carbohydrates in dietary obese mice: improvement by combination treatment with n-3 polyunsaturated fatty acids and calorie restriction

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Background and aims: Metabolic flexibility is the capacity for the organism to adapt fuel oxidation to fuel availability and it is usually impaired in obese, insulin-resistant subjects. In this study, we attempted to validate the use of intragastric glucose gavage and indirect calorimetry (INCA) for the measurement of metabolic flexibility to carbohydrates in lean and dietary obese C57BL/6J mice. In addition, we used this approach to assess the effects of a combination treatment by n-3 polyunsaturated fatty acids (PUFA) and 10% calorie restriction (CR) on metabolic flexibility in mice fed a high-fat diet.
Materials and methods: Female C57BL/6J mice were fed either a standard chow (STD) or corn oil-based high-fat diet (cHF; lipids ~35% wt/wt) from

the weaning (4 wk of age) until 7 mo of age (n = 6-9). Metabolic flexibility was assessed as a maximal change in respiratory quotient (ΔRQ) measured by INCA during a 4-hr period following a glucose load (0.45 ml of 50% D-glucose) administered by intragastic gavage to overnight (~12 hr) fasted animals. In the second experiment, 3-mo-old male C57BL/6J mice were habituated for 2 wk to the cHF diet, followed by differential dietary treatments (n = 8-9) for 5 wk: (1) cHF, *ad libitum*; (2) cHF with n-3 PUFA concentrate (EPAX 1050TG; EPAX, a.s., Lysaker, Norway) replacing 15% of dietary lipids, *ad libitum* (cHF+F); (3) cHF, 10% CR (cHF+CR); or (4) cHF+F, 10% CR (cHF+F+CR). Changes in plasma NEFA in response to fasted to fed transitions were also analyzed.

Results: In the first experiment, cHF feeding for 6 mo induced a weight gain of 31.7 ± 2.5 as compared to a gain of 10.8 ± 0.5 g in the STD mice (body weight: cHF, 45.8 ± 2.4 vs. STD, 25.0 ± 0.6 g; $p<0.001$). Although there were no differences between cHF and STD mice in RQ following overnight fasting (cHF, 0.746 ± 0.010 vs. STD, 0.773 ± 0.008; p=0.057), cHF-fed mice showed a ~2-fold lower increase in RQ in response to the glucose load as compared to STD controls (ΔRQ; cHF, 0.062 ± 0.006 vs. STD, 0.120 ± 0.010; $p<0.001$), suggesting impaired metabolic flexibility. In the second experiment, compared with cHF mice, all the treatments tended to prevent body weight gain (cHF > cHF+F > cHF+CR > cHF+F+CR), while a significant reduction was found only in mice subjected to the combination treatment (cHF, 28.7 ± 0.7 vs. cHF+F+CR, 25.8 ± 0.4 g; $p<0.001$). This treatment was also the most effective in elevating RQ in response to glucose (ΔRQ; cHF, 0.086 ± 0.002 vs. cHF+F, 0.095 ± 0.006 vs. cHF+CR, 0.096 ± 0.005 vs. cHF+F+CR, 0.107 ± 0.006; $p<0.05$ cHF vs. cHF+F+CR). At the same time, cHF+F+CR induced a most prominent decrease in plasma NEFA following fasted (cHF, 0.89 ± 0.05 vs. cHF+F+CR, 1.13 ± 0.04 mmol/l) to fed (cHF; 0.59 ± 0.09 vs. cHF+F+CR, 0.43 ± 0.04; $p<0.001$ cHF vs. cHF+F+CR mmol/l) transition, while the other treatments were less effective.

Conclusion: Metabolic inflexibility to carbohydrates could be demonstrated using intragastric glucose gavage and INCA in dietary obese mice. Combination treatment using n-3 PUFA and 10% CR preserved metabolic flexibility better than any of these treatments applied separately.

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Cross-linked dairy protein attenuates postprandial glucose and insulin levels and increases fullness in healthy young men

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Background and aims: Food induces many signals in the gastrointestinal tract, important for digestion, satiety and further systemic responses. The properties of foods are determined by their chemical composition and physical properties. Protein has the highest satiety value of among the different macronutrients. Furthermore, the textural properties of a single protein can be tailored by cross-linking enzymes e.g. transglutaminase (Tg). However, little is known about the effects of modified protein structures on these responses. Our aim was to examine the effects of intact and transglutaminase (TG) cross-linked dairy protein on postprandial hormonal, metabolic and appetitive responses.

Materials and methods: The study had a randomized repeated-measures crossover design. All participants tested each test product with a minimum of 2 d separating the individual test days. Eight healthy males (24.0±0.82 y, 23.3±0.5 BMI kg/m^2) consumed with 400 ml water isocaloric (850 kJ) and isovolumic (400 ml) test product containing either 50 g whey (Wh), casein (Cas) or casein protein cross-linked with transglutaminase (Cas-TG) in a randomized order. Blood samples were drawn for plasma glucose, insulin, CCK, GLP-1 and PYY analysis for 240 min. Appetite ratings were assessed at concomitant time points using visual analogue scales.

Results: Glucose levels significantly decreased in the first hour and returned close to baseline at the end of sampling at 240 min. Cas and Wh were more potent in lowering glucose levels than Cas-Tg. Release of insulin, GLP-1, PYY and CCK differed significantly in response to the three protein meals, with the highest insulin release 30 min after Wh. The insulin response to Cas-Tg was attenuated and peaked at 60 min. GLP-1 peaked at 15 - 30 min after Wh and 60 min after Cas, whereas the response to Cas-Tg was attenuated. PYY peaked after 30 - 60min, with the highest levels after Cas and the lowest after Cas-Tg. CCK increased similarly in the first 15 min after the Wh or Cas meal, while the release after Cas-Tg was lower, but more sustained. The feeling of fullness was the strongest after the Cas-TG compared to Cas and Wh.

Conclusion: Cross-linked milk protein attenuates the release of the GI hormones affecting plasma glucose and insulin levels and enhances fullness. The modification of protein texture could thus offer a tool for optimizing the postprandial glucose and insulin metabolism and postprandial appetite and thereby promote weight management and glycaemic control in obese and DM 2 patients.

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Effects of different types of meal on the expression on oxidative mitochondrial genes in skeletal muscle of healthy subjects

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Background and aims: Recent studies have shown an impaired mitochondrial oxidative capacity in skeletal muscle of individuals with insulin resistance, suggesting that mitochondrial dysfunction may be a primary, genetically determined defect. There is also evidence that mitochondrial dysfunction may be a consequence of adverse environmental factors, such as sedentary lifestyle and over nutrition. To evaluate the impact of acute administration of different dietary fat on the expression of muscle mitochondrial genes regulating replication and function in healthy subjects.

Material and methods: Six healthy subjects (3F/3M; age 29±3 years; BMI 25.0±3 Kg/m^2) received in a random order a test meal with the same energy content (970 Kcal) but different composition in macronutrients and quality of fat: Mediterranean meal (M) (Lipid 30% of which 6% saturated), SAFA meal (Lipid 67% of which 36% saturated) and MUFA meal (Lipid 63% of which 37% monounsaturated). At fast and after 180 min, a fine needle aspiration (FNA) was performed from the vastus lateralis muscle for determination of mithocondrial gene expression by quantitative PCR.

Results: M meal was associated with a significant increase ($p<0.05$) of transcription factor PPARs expression levels, whereas expression of regulator genes PGG1α and PGG1β remained substantially unchanged. No change was detected in the expression of COX5b, COX2 and GLUT 4 genes. After MUFA meal, no modification was observed in the expression of PPARs, but a significant increase of PGC1α (p=0.07) and PGC1β ($p<0.001$) gene expression was observed. COX5b e COX2 gene expression remained unchanged whereas GLUT4 gene expression increased significantly ($p<0.05$). After SAFA meal, PGG1α, PGG1β, and PPARs gene expression were unchanged whereas COX2 expression decreased by 38 % ($p<0.02$), COX5b decreased by 31% (p=0.07), and GLUT4 decreased by 11% ($p<0.05$).

Conclusion: The present data indicate that mitochondrial gene expression in skeletal muscle of healthy subjects can be modulated by acute changes of nutrient intake. Dietary fat have a differential impact on the gene transcriptional profile since saturated fat, but not monounsaturated, downregulate the expression of genes involved in glucose transport and substrate oxidation.

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Dose-dependent anti-obesity and anti-diabetic effects of synbiotics in high-fat fed C57BL/6J mice

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Background and aims: We have recently shown that plants rich in polyphenols (with antioxidants and prebiotic effects) administered alone or together with the probiotic dietary bacteria Lactobacillus plantarum (Lp) exerts anti-obesity and anti-diabetic effects in HFD-fed C57BL/6J mice, a model of human obesity and insulin resistance. For instance, we observed an almost complete prevention of body weight gain, as an effect of decreased adiposity, in groups receiving the supplemented diets. In addition, we observed an inhibited inflammatory activity in mice receiving the combination of supplement. Based on these results where the dramatic effects were attained with relatively high polyphenol concentration, we here aim to evaluate the dose-response relationship for the studied plant-associated polyphenol, both in absence and presence of probiotic bacteria.

Materials and methods: C57BL/6J mice were fed high fat diet supplemented with different concentrations of plant-associated polyphenols (pph 0, 0.4, 2 and 4%) without or with Lactobacillus plantarum (Lp 3 x 10^9 cfu/ml drinking water) for 12 weeks. Body weight, body fat and metabolic blood parameters were registered throughout the study. Oral glucose tolerance (OGTT) was performed in the end of the study. At the time of sacrifice, plasma and tissues were collected.
Results: Supplement of the plant-associated polyphenol, without or with probiotics, decreased body weight gain (pph: 0%:10.2±0.4, 0.4%:9.7±0.7, 2%:7.8±0.5, 4%:5.8±0.4g, pph+Lp: 0%:10.5±1.5, 0.4%:10.0±0.8, 2%:9.4±1.2, 4%:5.7±0.5g) and adiposity (pph: 29.2±1.3, 28.7±1.5, 25.7±1.6, 20.4±0.8%, pph+Lp: 31.2±3.3, 31.3±2.2, 26.1±2.6, 17.7±0.7%) in a dose-dependent manner. Dose-dependent decrease was also observed in the liver weights (pph: 1.2±0.0.4, 1.2±0.05, 1.1±0.0.5, 0.9±0.03g, pph+Lp: 1.2±0.13, 1.0±0.06, 1.2±0.07, 0.8±0.04g), and more pronounced in mice receiving the combination of polyphenols and probiotics than in mice receiving polyphenols alone, indicating a synbiotic beneficial effect. Also, mice fed the combination of supplements showed decreased fasting plasma glucose (pph+Lp: 6.9±0.7, 5.9±0.4, 6.2±0.0.6, 4.6±0.2 mM) with increasing concentration of polyphenols, although only the highest concentration (4%) of polyphenols was significantly decreased compared to ctrl.
Conclusion: High-fat diet supplemented with different concentrations of a polyphenol-rich plant powder, without or with addition of probiotics, gives dose-dependent beneficial effects on body weight, adiposity and glucose control. The observed advantageous effects are more pronounced in the presence of Lactobacillus plantarum, indicating a synbiotic effect.
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Effects of supplementation with red wine polyphenols on inflammation, mitochondrial function and oxidative stress muscle
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Background and aims: The mechanisms responsible for skeletal muscle insulin resistance (IR) remain incompletely understood. However, chronic low-grade inflammation, oxidative stress and mitochondrial alterations have been suggested to take part in the development of IR. Recent studies have found that polyphenolic compounds found in red grape have interesting properties against IR. Thus, the aim of this study was to investigate the impact of a supplementation with phenolic compounds from red grape marc in a genetically modified mouse (CD4dnTGFbRII; TGF) presenting a chronic inflammation compared to C57BL/66 (CTL).
Materials and methods: 14 TGF mice and 16 CTL mice were randomized between a control group (placebo, PL) and a group supplemented with polyphenols (PP) administered in drinking water (50mg/kg/jour) for a period of 4 weeks. The mice were sacrificed and their muscles taken for the study of muscle inflammation (RT-PCR), oxidative stress (mitochondrial carbonylated proteins) and fiber size (atrophy marker). The mitochondrial respiration was measured on mitochondria isolated from skeletal muscle.
Results: In TGF mice, the PP decreased muscle atrophy by 20% (p<0,0001) without reducing the expression of mRNA of inflammation markers (TNFalpha, OAS1, OAS2). In CTL mice, the PP increased the amount of TNFalpha mRNA by 700% (p=0,001) and decreased fiber size by 12% (p<0,0001). In CTL and TGF mice, the PP supplementation decreased mitochondrial respiration (TGF mice: 387 ± 35 vs 276 ± 33 nmol O / min / mg prot; CTL mice 277 ± 38 vs 178 ± 41 nmol O/min / mg prot, comparison PL vs PP by Anova p=0,009) without changing the amount of ATP synthesized. We also observed a decrease in the level of carbonylated mitochondrial proteins with PP supplementation in both strains of mice (comparison PL vs PP by Anova p=0,03).
Conclusion: The PP supplementation decreased mitochondrial oxidative stress and improved mitochondrial function in muscle of inflammatory and control mice. They also reduced muscle atrophy in inflammatory mice. Paradoxally, the PP increased the markers of inflammation and decreased fiber size in CTL mice. Further research should be persued to evaluate the effects of PP on muscle mass preservation in inflammatory states.
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Reduction of glycaemic index enhances levels of midregional-pro-atrial-natriuretic peptide: evidences for gut-heart-axis?
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Background and aims: Reduction of glycemic index is an effective strategy for prevention of hypertension and CVD events in the subjects with metabolic syndrome and T2DM. Atrial natriuretic peptide (ANP) is a potent natriuretic and vasorelaxant hormone that is secreted mainly by cardiomyocytes and plays contributory roles in cardiovascular homeostasis. In the subjects with Metabolic Syndrome (MS) circulating level of N-terminal natriuretic peptides are decreased for unknown reason. The midregional-pro-atrial-natriuretic peptide (MR-proANP) is a stabile fragment of the ANP precursor proANP, which is co-secreted with mature ANP from cardiomyocytes. We hypothesized that reduction of glycemic index by alpha-glucosidase inhibitor acarbose may modulate MR-proANP levels.
Materials and methods: Subjects with MS (n=28) were studied in the double blind, placebo controlled, crossover intervention study. Interventions with acarbose (3x100 mg/d) or placebo for 12 weeks (with a respective 12-week washout period) were performed. Changes in MR-proANP, postprandial glucose/insulin responses during liquid meal challenge test, body weight, and insulin sensitivity in the euglycemic clamp were assessed. Furthermore, in a cohort of normotensive non-diabetic subjects (n=46), the effect of insulin application on MR-proANP was analyzed during a hyperinsulinemic-euglycemic clamp.
Results: Fasting MR-proANP increased after 12 weeks of acarbose treatment (p=0.001). Acarbose decreased postprandial insulin and glucose concentrations (p=0.0001 and p = 0.024, respectively). Changes in MR-proANP levels correlated negatively with changes in postprandial insulin (r= -0.53, p<0.0001). No effects on body weight and insulin sensitivity were observed. Exogenous insulin suppresses plasma levels of MR-proANP (p<0.001).
Conclusion: Reduction of glycemic index by acarbose increases MR-proANP levels in subjects with MS. Moreover modulation of insulin levels has a strong effect on circulating MR-proANP. These observations provide a novel link between postprandial metabolism and hormonal heart action.
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Rose hip exerts anti-diabetic effects via a mechanism involving downregulation of the hepatic lipogenic program
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Background and aims: In the recent decades there has been a dramatic increase in the prevalence of obesity and type 2 diabetes worldwide. Both type 2 diabetes and obesity are strongly associated with non-alcoholic fatty liver disease, the most common liver disease worldwide. Rose hips are a rich source of antioxidants such as ascorbic acid, phenolic compounds and carotenoids. Recently it was shown that administration of an acetone extract from fruit and seeds from *Rosa canina* prevented body weight gain in mice fed a normal chow diet. The aim of this study was to explore the beneficial metabolic effects of rose hip in greater detail and to elucidate some of the mechanisms underlying the observed anti-diabetic effects. We used the high-fat fed C57BL/6J mouse which is a model for obesity, impaired glucose tolerance and early type 2 diabetes.
Materials and methods: Long-term metabolic effects were investigated in this mouse model following administration of powdered rose hip together with high-fat diet to lean mice. Parameters related to obesity and glucose tolerance were monitored, and livers were examined for lipids and expression of genes and proteins related to lipid metabolism and gluconeogenesis.
Results: A supplement of rose hip was capable of preventing the increase in body weight imposed by a high-fat diet in the C57BL/6J mouse (7.1±0.4 vs. 17.1±1.1g, p<0.001). The decreased body weight gain mirrored a lower body fat content, measured by dexa-scan technique. Lower basal levels of in-

sulin (91±15 vs. 351±99 pM, p<0.001) and glucose (5.5±0.3 vs. 9.4±0.5 mM, p<0.001) together with a reduced insulin response (AUC; 132531±19516 vs. 40427±7143, p<0.001) following an OGTT showed improved glucose tolerance in mice fed a supplement of rose hip compared to control mice. Hepatic triacylglycerol accumulation was reduced in mice fed rose hip compared to control (26±3 vs. 66±5 mg/g liver, p<0.001) and the expression of several lipogenic proteins was downregulated, whereas AMPK and other proteins involved in fatty acid oxidation were unaltered. Further, rose hip intake lowered plasma cholesterol (4.9±0.1 vs. 6.6±0.3, p<0.001) via a mechanism not involving altered gene expression of sterol regulatory element binding protein 2 and HMG-CoA reductase.
Conclusion: Taken together, these data show that a dietary supplement with rose hip prevents the development of a diabetic state in the C57BL/6J mouse. Downregulation of the hepatic lipogenic program appears to be at least one mechanism underlying the anti-diabetic effect of rose hip.
Supported by: Swedish Research Council, Swedish Diabetes Association, Novo Nordisk

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Vegetarian diet improves plasma concentrations of adipokines and oxidative stress markers more than conventional diabetic diet in subjects with type 2 diabetes
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Background and aims: Trials using vegetarian diet in subjects with type 2 diabetes (T2D) have shown greater improvement in HbA1c compared to conventional diabetic. We sought to investigate the effect of vegetarian diet on plasma concentrations of adipokines and oxidative stress markers.
Materials and methods: Subjects with T2D (n=74) were randomly assigned to experimental group (EG, n=37) following vegetarian diet or control group (CG, n=37) following conventional diabetic diet with the same caloric restriction. Participants were examined at baseline, 12 weeks of diet intervention and 24 weeks (second 12 weeks of diet were combined with aerobic exercise). Plasma concentrations of chosen adipokines and oxidative stress markers were measured using commercial kits.
Results: 43% of EG and 5% of CG participants reduced diabetes medication. Body weight decreased by 6.2 kg in EG and by 3.2 kg in CG (interaction between group and time p=0.001). Plasma concentrations of vitamin C increased in EG by 22% (p=0.002; group x time p=0.002). Superoxide dismutase increased in EG by 49% (p<0.001), whereas in CG it decreased by 30% (p<0.001; group x time p<0.001). Catalase increased in both groups (p<0.001 for EG and p=0.01 for CG). TBARS decreased in both groups (p<0.001 for both groups). Reduced glutathione increased in EG by 27% (p=0.02), whereas in CG it decreased by 11% (p=0.05; group x time p<0.001). Glutathione reductase decreased in EG by 42% (p<0.001) while glutathione peroxidase increased in CG by 20% (p<0.001) and glutathione transferase increased in both groups, more in CG (by 59% vs. 14% in EG; group x time p=0.003). Plasma concentrations of total adiponectin increased in EG by 19% (p=0.05; group x time p=0.02). HMW adiponectin increased in EG by 15% (p=0.02; group x time p=0.05). Resistin increased by 24% in CG (p=0.01; group x time p=0.005). Leptin decreased by 35% in EG (p=0.02; group x time p=0.05).
Conclusion: Vegetarian diet led to greater weight loss and reduction of diabetes medication and to greater improvement in plasma levels of adipokines and oxidative stress markers. Vegetarian diet could be a more convenient alternative in treatment of T2D.
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PS 88 Initiating and intensifying insulin therapy

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Negative attitudes towards insulin treatment in type 2 diabetes seems to be a rather temporal and benign phenomenon: Results of an observational longitudinal study
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Background and aims: Cross sectional findings indicate that negative attitudes towards insulin therapy are rather frequent in type 2 diabetes. These attitudes may be based on beliefs that the need for insulin therapy indicates a higher severity of diabetes and proves a failure of successful diabetes self-management. Worries about painful injections and the risk of hypoglycaemia or weight gain are also common. These negative attitudes may be one reason for the delay of insulin treatment initiation. In this observational longitudinal study with a three month follow-up the course of negative attitudes towards insulin treatment was analysed in three different groups of type 2 diabetic patients facing an intensification of diabetes treatment.
Materials and methods: The first subgroup was on insulin therapy at baseline (n = 57; age 56.0 ±8.9, disease duration 12.7 ±7.2 yrs, HbA1c 8.5 ±1.6%) and remained on insulin at follow-up. Of an initial 73 insulin-naïve patients, 44 were switched to insulin therapy (group 2: age 58.1 ±6.8, disease duration 7.7 ±5.0 yrs, HbA1c 9.1 ±1.7%) and 29 patients remained on an oral regimen (group 3: age 52.7 ±10.7 yrs, disease duration 5.3 ±4.6 yrs, HbA1c 8.3 ±1.4%). Barriers towards insulin therapy were measured using the Insulin Treatment Appraisal Scale (ITAS). As generic instruments of health related quality of life patients completed also the Problem Areas of Diabetes Questionnaire (PAID), the WHO-5 Well-Being Scale (WHO-5), the Centre for Epidemiologic Studies Depression Scale (CES-D) and the Trait Version of the State Trait Anxiety Inventory (STAI) at baseline and at a three month follow-up.
Results: In the three month follow-up HbA1c improved in all three groups (7.7 ±1.2% vs. 7.1 ±1.1% vs. 6.7 ±1.7%). The course of negative appraisal of insulin therapy was significantly different in the 3 groups (p=-003): It increased in patients remaining on an oral regimen (51.2 ±12.2 to 53.6 ±12.3), whereas the ITAS score decreased in patients who switched to insulin (:49.2 ±9.8 to 46.2 ±9.9) and patients who remained on insulin (45.8 ±8.3 to 44.5 ±8.0). More generic psychological variables like diabetes related distress, trait-anxiety or well-being showed an improvement in all three groups, but no significant differences between the groups. In patients who switched to insulin therapy the depression score improved significantly (16.1±10.2 to 11.9±9.8) compared to the groups who remained on oral medication (11.7 ±8.2 to 10.8 ±7.9) respectively who remained on insulin therapy (CES-D: 17.8 ±10.9 to 16.4 ±9.8 p=,045).
Conclusion: In type 2 diabetic patients who switched to insulin therapy negative appraisal of insulin therapy was reduced to the level of type 2 diabetic patients already treated with insulin. Patients remaining on an oral regimen increased negative appraisal towards insulin therapy. In summary this study shows that negative appraisal of insulin treatment is modifiable by the initiation of insulin therapy, indicating that features of "psychological insulin resistance" are a benign, temporary phenomenon. More meth-odological robust randomized studies are needed to corroborate this result further.
Supported by: Novo Nordisk

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Beginning insulin in people with type 2 diabetes mellitus in real life practice – 1-year results of the 4-year CREDIT Study
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Background and aims: CREDIT, a 314-centre, non-interventional study, investigates the effects of long-term glycaemic control with insulin treatment

on the risk reduction of cardiovascular events associated with type 2 diabetes mellitus (T2DM). Here, we present the 1-year findings of the CREDIT study in patients who initiated insulin at baseline.
Materials and methods: People with T2DM (n=3031) who had recently started insulin (basal, short-acting or premix insulin at the physician's discretion) were eligible for evaluation. Changes in therapy, metabolic control parameters and lipid profiles of 2734 people at baseline and at 1 year (9–18 months) after insulin initiation are described.
Results: Most people (75%) had the same insulin regimen at baseline and at 1 year (Table). Those starting with short-acting insulin regimen alone were more likely to have changed insulin regimen. Overall, the insulin dose increased from 19.7 ± 14.5 U/day at baseline to 34.4 ± 25.3 U/day at 1 year. Substantial reductions in HbA_{1c} (−1.8 %), fasting plasma glucose (FPG; −3.6 mmol/L) and postprandial PG (PPPG; −4.7 mmol/L) were observed; 32% of patients had HbA_{1c} <7.0 %. Symptomatic hypoglycaemia occurred in 20% of patients (2% experiencing at least one severe episode). Mean weight increased marginally by 1.7 ± 4.8 kg (from 80 ± 19 at baseline to 81 ± 18 at 1 year). LDL cholesterol levels were reduced slightly by −0.2 mmol/L (from 2.9 ± 0.9 to 2.7 ± 0.9 mmol/L) and triglycerides by −0.3 mmol/L (from 2.2 ± 2.4 to 1.8 ± 1.4 mmol/L). Mean HDL cholesterol levels were unchanged.
Conclusion: Although the results of the 1-year analysis of the CREDIT study are encouraging, the majority of patients remain above the HbA_{1c} target level of ≤7.0 % commonly advocated. The high FPG and PPPG levels indicate that there may be suboptimal insulin dose titration over the first year. In contrast to the substantial decrease in HbA_{1c}, the small decrease in LDL levels indicates that physicians focus on glucose control rather than on other aspects of secondary prevention.
Table

	Baseline (n=3031)	1-year follow-up (n=2734)	Change from baseline (n=2734)
Insulin regimen (%)*			
Basal	52	41	
Basal + short-acting	15	21	
Short-acting	7	3	
Premix	23	27	
Other	3	6	
HbA_{1c} (%)	9.5 ± 2.0	7.7 ± 1.4	−1.8 ± 2.1
FPG (mmol/L)	11.6 ± 3.7	7.9 ± 2.5	−3.6 ± 3.9
PPPG (mmol/L)	14.2 ± 4.6	9.6 ± 3.2	−4.7 ± 4.9

Data are % or mean ± SD; *No insulin at 1 year: 2.3%

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Fasting plasma glucose 6 to 12 weeks after starting insulin glargine predicts success in reaching HbA_{1c} ≤7.0% at weeks 24 to 28
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Background and aims: Adding insulin glargine, using a treat-to-target method, restores glycated hemoglobin A1C (A1C) to ≤7.0% for many patients with type 2 diabetes (T2DM) with inadequate glycemic control on oral agents, but some need further therapy to reach this goal. The purpose of this analysis was to assess whether early fasting plasma glucose (FPG) values predict glycemic control at week 24, and to help identify patients who may need prandial insulin after initiation of basal insulin.
Materials and methods: We analyzed patient-level data from 7 prospective, randomized controlled trials of insulin glargine with/without oral antidiabetic drugs in adults with T2DM with 24- or 28-week measurements and lab-measured FPG at week 6 or 8 and at week 12. These studies utilized strict, predefined insulin titration algorithms to achieve FPG concentrations ≤5.55 mmol/L.
Results: A total of 1036 patients (56% men; 81% white) had (mean±SD) age 56±10 y, duration of diabetes 8.4±5.9 y, baseline A1C 8.8%±1.0%. Mean A1C at endpoint was 7.03%±0.86%; mean FPG at endpoint was 6.67±1.9 mmol/L; 56% of patients reached A1C ≤7.0%. Mean FPG was 11.2±3.0 mmol/L at baseline, 7.33±2.2 mmol/L at week 6/8, and 6.77±1.9 mmol/L at week 12. Lower FPG at baseline was associated with lower A1C at week 24 (r=0.169, P<0.0001), but this correlation was stronger at week 6/8 (r=0.319, P<0.0001) and week 12 (r=0.317, P<0.0001). The figure shows percentage of patients reaching A1C ≤7.0% after having FPG in various ranges at baseline, week 6/8, and week 12. Patients with FPG ≥8.88 mmol/L at baseline had a likelihood of reaching A1C ≤7.0% similar to that of the whole population (55% vs 56%), but just 37% of those in this range at week 6/8 and 35% at week 12 did so. However, patients with FPG <8.88 mmol/L vs ≥8.88 mmol/L at week 12 had greater rates of symptomatic hypoglycemia (64.9% vs 51.8%). Laboratory FPG measurements correlated strongly with values self-measured at home (r=0.778, P<0.0001).
Conclusion: 1) Even a single FPG measurement (by laboratory or at home) can help identify persons starting insulin glargine who may need prandial therapy as well; 2) a value ≥8.88 mmol/L between weeks 6 and 12 indicates reaching target A1C ≤7.0% is unlikely and calls for individualized attention.
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Basal supported oral therapy (BOT) and risk of transition to intensified regimens: a retrospective cohort study
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Background and aims: After failure of oral antidiabetic drugs (OAD), treatment with long acting insulin plus oral antihyperglycaemic agents (basal supported oral therapy, BOT) is preferably recommended in type 2 diabetic (T2D) patients according to the consensus statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). We aimed to assess the persistence of T2D patients starting a BOT with either insulin glargine (GLA) or NPH insulin (NPH) until modification of insulin therapy.
Materials and methods: A retrospective cohort study was performed using claims data for ambulatory prescriptions within the German statutory health-insurance scheme, based on a representative sample of more than 80 % of community pharmacies. Patients on BOT with either GLA or NPH between 01/2003 and 12/2006 were included and followed up until 12/2007. Persistence was defined as the duration of time from initiation of BOT with GLA or NPH until modification of insulin therapy, with either premixed insulin (CT), bolus insulin alone (SIT) or bolus insulin added to basal insulin (ICT). Univariate and multivariate proportional hazards models were used to compare both cohorts.
Results: In total, 97,976 patients (61,053 GLA and 36,923 NPH) were included. Altogether, 44.1 % of GLA patients and 48.7 % of NPH patients modified initial BOT. On average, these patients stayed 373 days on BOT with GLA and 305 days on BOT with NPH (incidence rate per 100 person-years: 25.8 vs. 33.4). During the observation period 23.7 % of the patients switched to ICT, 17.6 % to CT, and 4.5 % to SIT. The risk of switching from BOT to one of these intensified insulin regimens was significantly higher for NPH compared to GLA patients (HR 1.25, 99 % CI 1.22-1.28). After adjustment for predefined covariables i.e., type of physician, region, insurance status, health insurance company, comedication, number of OADs, dose of basal insulin, the risk for NPH patients remained significantly higher (HR 1.17, 99 % CI 1.14-1.20).
Conclusion: T2D patients under BOT with GLA remain significantly longer compared to NPH before they have to be switched to more intensified regimens. This might also be of economic importance, since BOT causes less resource consumption than CT, SIT or ICT regimens as has been shown by other investigators.
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The STEPwise™ randomised, controlled, 48-week trial: intensifying treatment with stepwise addition of prandial insulin aspart, based on largest prandial glucose increment or largest meal, to once-daily basal insulin detemir in subjects with type 2 diabetes
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Background and aims: Adding bolus insulin doses step by step is one approach for intensifying treatment in patients on basal insulin when glycaemic

control is no longer maintained. The aim of this randomised, controlled, parallel-group, open-label, 48-week trial (STEPwise™) was to compare the effect on glycaemic control and safety endpoints of sequential addition of prandial insulin aspart (IAsp) to: (a) largest perceived meal (SimpleSTEP); or (b) meal with the largest post-prandial glucose increment (ExtraSTEP).
Materials and methods: 296 subjects with type 2 diabetes inadequately controlled on basal insulin+OADs (mean age 58.3 yrs; mean HbA_{1c} 8.8%; mean diabetes duration 12.3 yrs) underwent a 12-week run-in and were transferred to once-daily bedtime insulin detemir with continuation of their previous stable pre-trial OAD regimen and optimisation of basal insulin doses. Subjects with HbA_{1c} ≥7% after run-in were randomised to one of the two groups, and sulphonylureas were discontinued. Bolus insulin titration was based on pre-meal glucose values for SimpleSTEP and post-meal glucose values for ExtraSTEP. After 12 weeks' treatment with the 1-IAsp regimen (Period 1), subjects not at HbA_{1c}<7% received a second IAsp titrated bolus at the next largest meal or uncovered meal with largest post-prandial glucose increment for an additional 12 weeks (Period 2); subjects with HbA_{1c} <7% continued with one IAsp injection. At 24 weeks, subjects with HbA_{1c} ≥7% received IAsp at a second/third meal and subjects with HbA_{1c} <7% continued on the 1- or 2-IAsp regimens for 12 more weeks (Period 3).

	SimpleSTEP (*n*=150)			ExtraSTEP (*n*=146)			End of trial estimated difference, 95% CI, *p*-value
Efficacy [mean (SE)]	Period 1	Period 2	Period 3	Period 1	Period 2	Period 3	
HbA_{1c} (%)	8.3 (0.06) (End of run-in: 8.7%)	7.8 (0.08)	7.6 (0.08)	8.4 (0.07) (End of run-in: 8.9%)	7.9 (0.08)	7.9 (0.08)	0.06 [-0.17, 0.29], *p*=0.606
FPG (mmol/L)	8.2 (0.26)	7.7 (0.25)	7.8 (0.26)	7.9 (0.25)	7.6 (0.25)	7.5 (0.26)	-0.27 [-0.99, 0.44], *p*=0.456
Weight change trial end (kg) [mean (SD)]	2.7 (3.9)			2.0 (3.8)			-0.67 [-1.60, 0.27], *p*=0.162

Results: At study end, both groups showed significant improvements in HbA_{1c} (~1.2%) with no difference between regimens. HbA_{1c} decreased by ~0.5% in Period 1, by a further ~0.5% in Period 2, and by ~0.2% in Period 3 in both groups. The overall rate of hypoglycaemia was low.
Conclusion: Improvement in glycaemic control with a low risk of hypoglycaemia can be achieved by the stepwise addition of insulin aspart to insulin detemir based on perceived meal size or measured post-prandial glucose increments.
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Stepwise intensification of prandial insulin versus basal–bolus insulin therapy in patients with type 2 diabetes mellitus
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Background and aims: Insulin therapy intensification is important for patients with poorly controlled type 2 diabetes mellitus (T2DM). This international, randomized, parallel-group, non-inferiority study compared stepwise addition of prandial insulin with a basal–bolus regimen.
Materials and methods: A total of 811 patients (mean ± standard deviation [SD] age: 58.6 ± 8.9 years) with T2DM poorly controlled on basal insulin (HbA_{1c} 9.1 ± 1.4%; fasting plasma glucose [FPG] 9.7 ± 3.3 mmol/L [174 ± 59 mg/dL]) were switched to insulin glargine (GLAR) for 6 months, and continued oral therapy. At 6 months, mean GLAR dose was 36 U/day and mean HbA_{1c} was 8.3%. Patients with HbA_{1c} >7% and FPG <6.7 mmol/L (<120 mg/dL) (n=476) were then randomized to: Group 1) GLAR + metformin (MET) + 3× insulin glulisine (GLU); Group 2) GLAR + MET + 1–3× GLU; or Group 3) GLAR + MET + sulfonylurea + 1–3× GLU for 12 months. Patients in Group 1 received GLU at each meal; patients in Groups 2 and 3 injected GLU at the meal with the highest postprandial plasma glucose (PPPG), with two further doses added at Months 4 and 8 if HbA_{1c} was >7% (target PPPG 6.1–8.9 mmol/L [110–160 mg/dL]). Initial GLU dose was half the meal PPPG and titrated according to an algorithm based on the PPPG. Non-inferiority of Group 2 to Group 1 was concluded if the 95% confidence interval (CI) upper limit for the HbA_{1c} difference was ≤0.4%.
Results: The adjusted difference of Group 2 versus Group 1 for the per-protocol population (PP; primary analysis) crossed the non-inferiority margin (0.228 [95% CI: −0.018, 0.473]) but did not for the intent-to-treat population (ITT; secondary analysis; 0.122 [95% CI: −0.114, 0.358]). In both the PP and ITT, Group 1 was not superior to Groups 2 or 3 (Table). At endpoint, the GLAR dose was similar in all three groups with mean doses of 37, 40 and 40 U/day for Groups 1, 2 and 3, respectively. The mean GLU dose at endpoint was 29, 20 and 17 U/day, respectively; 33 and 40% of patients remained on one GLU injection in Groups 2 and 3, respectively. The incidence of symptomatic hypoglycaemia was highest in Group 3 and lowest in Group 2, although between-group differences were not significant (Table). Mean change in body weight was slightly increased in all three groups but the increase was significantly lower in Group 2 compared with Group 1 (Table).
Conclusion: Stepwise intensification of GLU added to GLAR showed efficacy similar to a basal–bolus approach with less symptomatic hypoglycaemia and significantly less weight gain, although non-inferiority was not achieved.
Table

	Group 1 (n=144)	**Group 2 (n=197)**	**Group 3 (n=123)**
HbA_{1c} (%; per protocol)	8.5 ± 1.1	8.4 ± 1.1	8.3 ± 1.1
Randomization	7.7 ± 1.2	7.9 ± 1.2	7.9 ± 1.3
Endpoint	−0.72 ± 1.25	−0.47 ± 1.05	−0.40 ± 1.11
Change			
Symptomatic hypoglycaemia (mean events/pt year)	4.36	4.23	5.46
Body weight (kg)	82.7 ± 15.6	81.6 ± 15.8	83.6 ± 17.0
Randomization	84.7 ± 15.1	82.9 ± 16.0	85.5 ± 17.5
Endpoint	+2.03 ± 3.21	+1.30 ± 3.17*	+1.90 ± 3.38
Change			

*p<0.05 versus Group 1

Supported by: sanofi-aventis

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Premeal injection of rapid-acting insulin reduces postprandial glycaemic excursions, a randomised controlled trial
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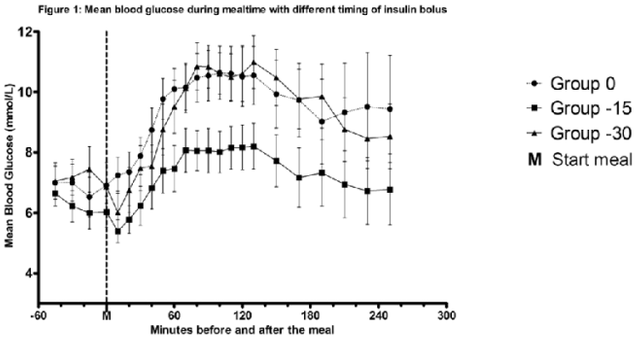
Background and aims: To assess the effect of three premeal timings of rapid-acting insulin on postprandial glucose excursions in patients with CSII treated type 1 diabetes.
Materials and methods: 10 subjects (3 females and 7 males) with T1DM participated. Mean (± SD) age was 45.5 ± 12.09 years, HbA1c 8.55 ± 1.50%, duration of diabetes 23.8 ± 7.81 years and duration of CSII therapy 8.5 ± 6.10 years. Patients were served an identical breakfast on three study days. Insulin aspart was randomly administered at 30, 15 or 0 minutes before the meal. All patients started the study with admission blood glucose between 3.5 and 7.8 mmol/L. Blood was sampled for glucose determination from one hour before the meal until four hours after.
Results: The area under the curve was significantly lower in the -15 group (0.41 ± 0.51 mmol/L/min) compared to the -30 group (1.89 ± 0.72 mmol/L/min, P = 0.029) and 0 group (2.11 ± 0.655 mmol/L/min, P = 0.030). The maximum blood glucose excursion was also lower in the -15 group (4.77 ± 0.52 mmol/L) compared to the -30 (6.48 ± 0.76 mmol/L, P = 0.025) and 0 group (6.93 ± 0.76 mmol/L, P = 0.022). The peak blood glucose level was significantly lower in the -15 group (9.26 ± 0.72 mmol/L) compared to the -30 group (11.74 ± 0.80 mmol/L, P = 0.007) and the 0 group (12.29 ± 0.93, P=0.009). Time spent in the 3.5 to 10 mmol/L range was highest in the -15 group (224.5 ± 25.0 min) and this difference was significant in comparison with the 0 group (90.5 ± 23.2 min, P=0.001) but not when compared to the -30 group (182.5 ± 28.2 min, P=0.212). There was no significant difference between the occurrences of glucose levels <3.5mmol/L between groups (P=0.901).
Conclusion: Administration of rapid acting insulin analogues at 15 minutes before mealtime results in lower postprandial glucose excursions and more time spent in the 3.5-10.0 mmol/L glucose range, without increased risk of hypoglycaemia.



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Clinical outcomes after basal insulin initiation correlate with baseline oral antidiabetic drug therapy: a pooled analysis of clinical trial data

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Background and aims: This analysis evaluated the association between baseline oral antidiabetic drug (OAD) therapy and clinical outcomes after insulin initiation.
Materials and methods: The analysis included data from 11 prospective randomized controlled trials of insulin glargine (without prandial insulin) with/without OADs in adults with type 2 diabetes; 2171 patients received insulin glargine. These studies used strict, predefined insulin titration algorithms to achieve fasting glucose concentrations ≤5.55 mmol/L. Study duration varied from 24 to 48 weeks; outcomes for the pooled analysis were assessed at week 24. Statistical analysis compared patients taking 0 or 1 OAD at baseline (low use; 1.8% and 45.2% of patients, respectively) with those taking 2 OADs (52.2%), and patients on metformin (MET) only (8.5%) with those on sulfonylurea (SU) only (36.5%) or MET + SU (49.9%).
Results: Mean age was 58.6 years, 55.6% were male, and 88.3% were white. At week 24, patients with low baseline OAD use and those taking only MET had significantly greater A1C reductions (Table). Weight gain from baseline to week 24 was not significantly different based on the number or type of baseline OADs. However, patients with low baseline OAD use had significantly lower rates of symptomatic hypoglycemia vs those taking 2 OADs (P=0.0009), and those taking only MET had lower rates than those taking SU or MET + SU (P<0.0001) despite higher insulin doses (53 vs 37.5 vs 38.8 U).
Conclusion: Patients adding insulin to 0 or 1 baseline OAD, who may be in earlier stages of diabetes, showed a greater reduction in A1C with lower risk of hypoglycemia than those taking 2 OADs. The more favorable results of adding insulin to baseline MET vs SU or combined therapy support the Tier 1 recommendations of the current algorithm of the American Diabetes Association/European Association for the Study of Diabetes.

Table: A1C and Baseline (BL) OAD Use

BL OAD	BL A1C, %[a]	Week 24 A1C, %[a]	Δ in A1C From BL to Week 24, %[b]	Week 24 A1C ≤7.0%, n (%)
0/1 OAD	8.87 (1.07)	7.05 (0.97)	-1.83 (0.05)	558 (54.7)
2 OADs	8.68 (1.02)	7.04 (0.89)	-1.68 (0.03)	643 (56.7)
P value[c]			0.0198	0.0541
MET only	9.08 (1.28)	6.87 (0.93)	-1.98 (0.07)	126 (68.1)
SU only	8.84 (1.02)	7.11 (0.99)	-1.69 (0.06)	399 (50.4)
MET + SU	8.67 (1.03)	7.04 (0.89)	-1.69 (0.03)	646 (56.8)
P value[c]			0.0009	<0.0001

[a]Mean (SD); [b]Least squares mean (SE); [c]From analysis of covariance model.

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The DURABLE Trial: comparing durability of lispro mix 25 vs glargine

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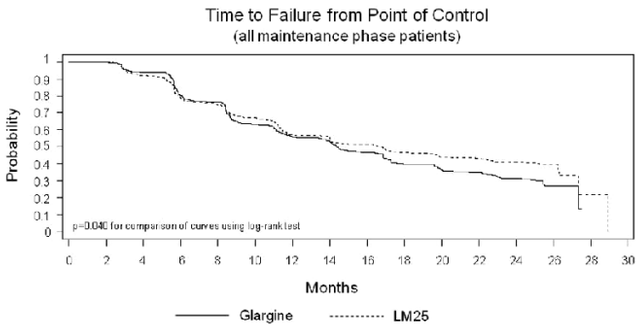
Background and aims: To evaluate if adding twice-daily (bid) insulin lispro mix 25 (LM25: 25% lispro/75% insulin lispro protamine suspension) to oral antihyperglycaemic medications (OAMs) results in longer durability of glycaemic control than adding once daily (qd) insulin glargine (G). Durability was defined as duration of time patients (pts) maintained hemoglobin A1c (HbA_{1c}) goal (HbA_{1c} ≤7.0% or HbA_{1c} >7.0% but an increase of <0.4% from the last HbA_{1c} ≤7.0%).
Materials and methods: A total of 2091 pts with type 2 diabetes (T2D) aged 30-80 yrs, HbA_{1c} >7.0% on at least 2 OAMs were randomised to either LM25 bid or G qd. OAMs were continued. Insulin was titrated to fasting plasma glucose (FPG) and pre-evening meal plasma glucose <6.1 mmol/L for LM25, and FPG ≤5.5 mmol/L for G. All pts had up to 6 months (initiation phase) to achieve HbA_{1c} ≤7.0%, after which, pts with HbA_{1c} ≤7.0% advanced into the maintenance phase for up to an additional 2 years (total = 30 month study). Pts were discontinued from maintenance phase if HbA_{1c} was >7.5%. The primary objective was duration of time at HbA_{1c} goal, from when pts first attained HbA_{1c} goal.
Results: Of the 900 pts on LM25 and 918 pts on G who completed the initiation phase, 473 and 419, respectively, had an HbA_{1c} ≤7.0% at 6 months and continued into the maintenance phase. Baseline characteristics were similar across therapies except for age (LM25: 59±9 yrs; G: 57±9 yrs). The median time at HbA_{1c} goal was 16.8 months (95% CI, 14.0-19.7) for LM25 and 14.4 months (95% CI, 13.4-16.8) for G (p=0.040, log-rank test). At endpoint, HbA_{1c} goal was maintained for 202 (43%) pts on LM25 and 147 (35%) pts on G (p=0.006). Compared to pts unable to maintain HbA_{1c} goal, pts on LM25 who maintained HbA_{1c} goal throughout the study had lower baseline HbA_{1c} (p=0.043). Pts on G who maintained goal had: shorter duration of diabetes; lower baseline HbA_{1c}, mean post-meal plasma glucose (PG), and mean PG; and higher 1,5-anhydroglucitol (p<0.05 for all). There was no difference in the rate (episodes/patient/year) of overall (LM25 19; G 18), nocturnal (LM25 6; G 9), or severe (LM25 0.02; G 0.02) hypoglycaemia. Incidence of serious adverse events was not different.
Conclusion: LM25 therapy resulted in statistically longer durability than treatment with G, though the clinical relevance of this difference is unclear. Patients with a lower baseline HbA_{1c} were more likely to maintain HbA_{1c} goal, which supports the concept of early introduction of insulin therapy in T2D. In both groups, the median time to starter insulin failure illustrates the progressive nature of T2D, necessitating continued diligence and advancement of therapy.



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Switching from premixed insulin to basal–bolus insulin glargine plus rapid-acting insulin: results of the ATLANTIC study

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Background and aims: This study evaluated the efficacy and safety of switching from twice-daily premixed insulin to basal–bolus glargine (GLAR) plus rapid-acting insulin in a 'real-world' clinical practice setting in Belgium and The Netherlands.
Materials and methods: This was a prospective, 6-month, multicentre, non-interventional, observational study. Adults with Type 2 diabetes mellitus (T2DM) could be enrolled if they were being switched from twice-daily premixed insulin to basal–bolus GLAR plus insulin glulisine (GLU; The Netherlands) or any rapid-acting insulin (Belgium) due to poor glycaemic control. The primary objective was the proportion of patients with HbA_{1c} <7% at Month 6. Secondary objectives included changes in mean HbA_{1c}, fasting plasma glucose (FPG), self-monitored blood glucose (SMBG), weight, insulin dose, fasting blood lipid profile (FBLP), safety (including the incidence of symptomatic nocturnal and severe hypoglycaemia) and treatment satisfaction (DTSQs and DTSQc).
Results: A total of 214 patients were included: mean ± standard deviation age 64.4 ± 9.8 years, diabetes duration 12.1 ± 7.8 years, weight 89.5 ± 17.2 kg. GLAR was initiated with GLU, regular human insulin or insulin aspart in 81.7, 8.9 and 8.5% of patients, respectively. At baseline, 3.3% had HbA_{1c} <7%, which increased significantly at Months 3 and 6 to 20.1 and 24.9%, respectively (Table). Significant reductions over 6 months were observed in mean HbA_{1c}, FPG and SMBG. The mean total premixed insulin dose prior to switching was 65.2 U/day and the starting doses of GLAR and rapid-acting insulin were 28.1 and 35.7 U/day, respectively. Mean GLAR and rapid-acting insulin doses were significantly higher at Month 6. Mean weight increased by 0.67 ± 4.8 kg at Month 6 (not significant). No statistically significant changes in FBLP were observed at Month 6, although there was a trend toward a reduction in triglycerides (−14 mg/dL; p=0.063). At Month 6, 33.9 and 27.8% of patients reported a reduction in the incidence of nocturnal and severe hypoglycaemia, respectively, compared to the initial visit (both p<0.0001). Increases in these events were reported by 1.8 and 2.5%, with the remaining patients reporting no change. Overall, 22 adverse events were reported by 19 patients, including nine episodes of hypoglycaemia, which were considered non-serious. Treatment satisfaction improved significantly at Month 6 (DTSQs +5.9 and DTSQc +10.4; both p<0.0001).
Conclusion: In a Belgian and Dutch clinical practice setting, patients with T2DM poorly controlled on premixed insulin experienced significant improvements in glycaemic control and treatment satisfaction, without a concomitant increase in hypoglycaemic events or weight, when switched to basal–bolus GLAR plus rapid-acting insulin.

Table

	Baseline	Month 3	Month 6	p value*
% of patients with HbA_{1c} <7% (95% CI)	3.3 (1.6–6.7)	20.1 (14.9–26.5)	24.9 (19.0–31.8)	p<0.0001
HbA_{1c} (%)	9.2 ± 4.5	7.7 ± 1.0	7.5 ± 0.9	p<0.0001
FPG, mmol/L (mg/dL)	10.4 ± 4.1 (187 ± 73)	8.4 ± 5.3 (152 ± 96)	7.8 ± 2.7 (140 ± 48)	p<0.0001
7-point SMBG, mmol/L (mg/dL)	10.8 ± 2.7 (195 ± 48)	8.5 ± 2.0 (153 ± 36)	8.5 ± 2.0 (153 ± 36)	p<0.0001
Weight (kg)	89.5 ± 17.2	89.8 ± 17.1	90.4 ± 17.6	NS
Insulin glargine dose (U/day)	28.1± 17.4	32.8 ± 16.8	34.7 ± 22.1	p<0.0001
Rapid-acting insulin dose (U/day)	35.7 ± 17.5	43.0 ± 20.0	43.8 ± 20.9	p<0.0001
Hypoglycaemia, reduction/no change/increase (%)* Nocturnal Severe	– –	29.4 / 66.1 / 4.4 25.7 / 70.9 / 3.4	33.9 / 64.2 / 1.8 27.8 / 69.8 / 2.5	p<0.0001 p<0.0001

Data are mean ± standard deviation unless otherwise stated. *Change from baseline to Month 3 and 6; CI=confidence interval; NS=not significant

Supported by: sanofi-aventis

PS 89 Short-acting insulins

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Pharmacokinetics of novel formulations of insulin analogues that provide a more rapid onset of action in diabetic miniature swine

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Background and aims: Commercial prandial insulin analog formulations have a more rapid onset of action than traditional regular human insulin preparations. A faster absorption profile is desirable to improve the timing of insulin release and reduce hypoglycemic events between meals. In this pre-clinical study, Insulin lispro (ILV), insulin aspart (IAV) and insulin glulisine (IGV) were re-formulated with safe excipients to improve their rate of subcutaneous absorption. The aim of this study was to evaluate the pharmacokinetic (PK) timing of these new rapid acting insulin analog formulations.
Materials and methods: ILV, IAV and IGV were formulated in a similar manner to VIAject (containing EDTA and citrate) and were compared to their commercial preparations (insulin lispro (IL), insulin aspart (IA), and insulin glulisine (IG)) in diabetic miniature swine (DMS). DMS were given a dose of 0.25 U/kg in lieu of their daily porcine insulin injection. Immediately following dosing, the swine were fed 500 g of their normal swine diet. Blood glucose and plasma insulin were sampled at -30, -20, -10, 0, 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, 240, 300, 360, 420, and 480 min. post dose. Insulin analog plasma levels were measured by ELISA technique.
Results: Results of timing related PK parameters Tmax and 1/2Tmax are shown in table below. In all cases, the new formulations ILV, IAV and IGV had significantly faster insulin absorption than their commercial counterparts, IL, IA and IG. Pharmacodynamics were consistent with the PK.
Conclusion: Viaject, a formulation of RHI combined with these exipients has completed phase III trials as an improved prandial insulin and is currently being reviewed by the FDA. The phase III clinical trials showed that not only was insulin absorption more rapid than RHI and analog insulins, but there was also a significant beneficial decrease in weight gain and reduction in hypoglycemia compared to RHI. This information, coupled with results of the present study, suggest existing rapid acting analogs could be reformulated with these safe excipients and result in a safe and efficacious improvement of these prandial insulins.

Pharmacokinetic data

	ILV(n=8)	IL(n=10)	IAV(n=4)	IA(n=4)	IGV(n=5)	IG(n=11)
Tmax(min.)	23.0+/-5.5	30.0+/-6.1	17.5+/-2.5**	63.8+/-9.4	26.0+/-6.2	37.3+/-6.4
1/2Tmax(min.)	4.1+/-0.8**	15.1+/-3.4	6.9+/-1.8*	29.6+/-7.4	5.8+/-0.8*	9.4+/-1.1

*p<0.05, **p<0.01, +/-SEM

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Comparative pharmacokinetics and pharmacodynamics of high-dose human regular U-500 insulin versus human regular U-100 insulin in healthy obese subjects

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Background and aims: Human regular U-500 (U-500R) insulin is used in the US and UK in high-dose insulin-treated diabetes patients with the advantages of smaller injection volumes and fewer injections as compared to U-100 insulins/analogues. However, only a few pharmacokinetic (PK) and pharmacodynamic (PD) studies of U-500R have been conducted since its introduction in 1997. The primary aim of this study was to evaluate the relative exposure after 2 clinically relevant doses of U-500R vs U-100 human regular insulin (U-100R) in healthy obese subjects. Other comparative PK/PD responses were evaluated.
Materials and methods: Twenty-four healthy obese subjects (male/female 14/10; age [mean±SD] 39.6±12.1 years; body weight 98.1±12.9 kg; BMI 34.4±2.6 kg/m^2) participated in a single-centre, 4-period, 4-sequence, cross-over, randomised, double-blinded, euglycaemic clamp study. Following administration of 50-IU and 100-IU doses of each formulation, subjects underwent euglycaemic clamps up to 24 hours. Serum immunoreactive insulin

was measured for PK evaluation. Glucose infusion rates were recorded for PD analysis.

Results: Results for the 100-IU dose are shown in the table. While overall exposure (AUC from time zero to return to baseline, $AUC_{0-t'}$) was similar between formulations at both 50-IU and 100-IU doses, the U-500R peak concentration (C_{max}) was significantly lower than that for U-100R at both doses . The time-to-peak concentration (t_{max}) was significantly longer for U-500R at the 100-IU dose only. Overall effect (G_{tot}) for U-500R was similar to U-100R at both doses. Peak effect (R_{max}) was lower for U-500R vs U-100R at both doses. Time-to-peak effect (tR_{max}) was shown to be prolonged for U-500R vs U-100R at the 100-IU dose only. Time variables reflective of duration of action (early and late tR_{max50}, tR_{last}) were prolonged for U-500R vs U-100R at both doses.

Conclusion: While $AUC_{0-t'}$ was similar at both 50-IU and 100-IU, the peak concentration was significantly lower for U-500 at both doses. The PD results are generally consistent with the PK. Both U-500R and U-100R exhibited long time-to-peak effect and duration of effect, with U-500R being significantly longer for these parameters vs U-100R. For T2D patients requiring high-dose U-100R therapy, this may have important clinical implications for bolus/basal insulin calculations. The longer duration of effect of U500R compared to U100R suggests that multiple daily injections of U500R without use of a basal insulin may be a plausible treatment option for obese patients with type 2 diabetes; further study is required to determine the safety and efficacy of such an approach.

100-IU Dose		Human Regular U-500 Insulin	Human Regular U-100 Insulin	Ratio (†) or Difference (‡) of LS Means	90% CI
	$AUC_{0-t'}$ (pmol·min/L)	12300 (19)	12400 (22)	0.98†	(0.92, 1.05)
PK	**C_{max} (pmol/L)**	1020 (31)	1400 (28)	0.72†	(0.66, 0.78)*
	t_{max} (hr)	8.00 (0.50 - 8.00)	3.00 (1.00 - 8.00)	2.50¥	(0.00, 4.00)*
	G_{tot} (g)	621 (33)	586 (23)	1.09†	(1.01, 1.17)
	R_{max} (mg/min)	826 (37)	966 (22)	0.87†	(0.80, 0.95)*
	tR_{onset} (hr)	0.221 (77)	0.184 (78)	0.04‡	(-0.06, 0.14)
PD	**tR_{max} (hr)**	6.37 (25)	5.32 (22)	1.04‡	(0.38, 1.70)*
	Early tR_{max50} (hr)	2.02 (59)	1.51 (41)	0.60‡	(0.34, 0.87)*
	Late tR_{max50} (hr)	15.1 (16)	11.7 (16)	3.39‡	(2.54, 4.25)*
	tR_{last} (hr)	21.5 (11)	18.3 (15)	3.24‡	(2.29, 4.19)*

Parameters are expressed as geometric mean (CV%), except for t_{max}, expressed as median (range).

*p<0.05

¥Median difference

Supported by: Lilly USA, LLC

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More rapid onset and shorter duration of insulin exposure and action for 3 rapid insulin analogues coinjected with human hyaluronidase

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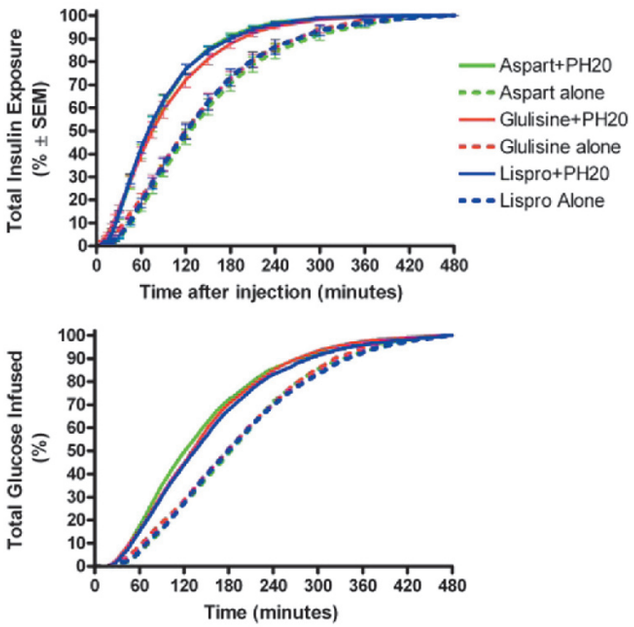
Background and aims: This study compared the pharmacokinetic (PK) and glucodynamic (GD) responses to 3 rapid-acting insulin analogs (glulisine, lispro and aspart) ± coinjected recombinant human hyaluronidase (PH20)

Materials and methods: A 6-way crossover glucose clamp study was conducted in 14 healthy volunteers [8 male, 6 female; mean age 34 (23-53); mean BMI 24.7 kg/m2 (21.1-27.0)]. Euglycemic clamps (8h at 90% FBG w/o basal insulin infusion) were performed following 0.15 U/kg doses of each study drug SC in a random order. Specificity and sensitivity of the insulin immunoassay used for PK analysis was slightly different for the three analogs; data are graphed as cumulative values to allow direct comparison of timing differences for exposure and action across the 3 analogs.

Results: The three analogs w/o PH20 had generally comparable insulin time-exposure and time-action profiles [time to 50% of total insulin exposure (AUC) were 124, 123 & 131 min; time to 50% insulin action (Gtot) were 183, 186 & 187 min, for glulisine, lispro and aspart; all P>0.2] although glulisine had modestly more rapid onset (time to 10%) of exposure (40 v. 47 & 48 min; P=.005 & .0004) and action (67 v. 76 & 76 min; P=.025 & .021). PH20 accelerated the absorption of all three rapid-acting analogs, resulting in more physiologic fast-in, fast-out profiles: insulin exposure in the 1st hr increased to 191%, 229%, and 246% control and after 2 hrs decreased by 43%, 54%, and 57%; glucose infused in the 1st 2 hrs increased to 157%, 161%, and 182% control, and after 4 hours decreased by 48%, 44%, and 50% for glulisine, lispro and aspart, respectively (all P<.0001). With PH20, all 3 analogs had comparable profiles (times to 50% of total insulin exposure were 79, 71 & 73 min and time to 50% insulin action were 135, 140 & 127 min, for glulisine, lispro and aspart respectively; all P≥.1 between analogs w/ PH20) and each was notably faster than any marketed products alone (all P<.0001). All injections were well tolerated; all adverse events (mild or moderate) were procedure related. Also shown in the figure are cumulative insulin exposure and action for regular human insulin (RHI) from data collected in a separate but similar study.

Conclusion: Coinjection with PH20 was well tolerated and accelerated the absorption of all 3 insulin analogs to a comparable degree, resulting in more rapid onset and shorter duration of both insulin exposure and action.



966

Dissolving microneedles for percutaneous delivery of insulin

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Insulin (Ins) chip having 300 2-layered dissolving microneedle (DM) arrays on 254mm^2 were prepared by microfabrication technology where water-soluble thread-forming polymer, chondroitin sulfate (Chon) was used as the base. The obtained Ins DM chips were evaluated as a new TDDS. The mean lengths of the DM were 473.3±3.7(SE)μm, though the mean length of Ins loaded space was 328.1±3.5μm from the top of DMs. The diameters of the basement were 301.6±1.6μm. One chip contained 5.7±0.04 and 7.1±0.7 IU Ins by HPLC analysis. After administration of Ins chip to the abdominal skin of dogs, plasma glucose levels were measured. Maximum hypoglycemic effect appeared at 1.0±0.0 h. The minimum glucose levels were 46.5±3.9 and 36.1±5.8% as compared to the pre-dose level. By comparing the AAC (area above the plasma glucose level *vs*. time curve) obtained after sc injection of Ins solution, 4.0 IU, relative pharmacological availabilities (RPA) were 54.9±5.9 and 56.3±5.1%. Plasma Ins levels were also measured by ELISA. C_{max}s were 148.2±57.2 and 163.8±44.2 mIU/mL and T_{max}s were 0.75±0.0 and 0.75±0.0h. Relative bioavailabilities (RBA) against sc Ins injection were 72.0±34.4 and 79.7±26.5 %. Fluorescence microscopy experiment using a DM chip containing FITC-insulin (F-ins) showed that DM was dissolved in the inserted epidermal site of the skin within 5 min and thereafter F-ins diffused both horizontal and vertical directions of the skin within 30 min. Histological study on the administered skin showed that there was no damage on the skin. As chip is made of Ins and Chon, safety is established. Ins DM chip is a useful TDDS and we want to proceed to clinical phase I study.

Keywords: Insulin microneedle chip
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No generation of insulin antibodies in subjects with impaired glucose tolerance treated with buccal spray insulin

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Background and aims: In patients with impaired glucose tolerance (IGT), upon implementation of life style changes and metformin, a third returns to normal glucose tolerance, a third continues with IGT and the rest goes on to develop clinical type 2 diabetes. An increased risk for cardiovascular disease occurs in the latter two groups even though there is no progression to diabetes. The implementation of treatment strategies to lower postprandial hyperglycaemia has been recommended by the IDF guidelines. A previous proof of concept study demonstrated that treatment with buccal spray insulin (Oral-lyn™) can be a valuable tool for managing subjects with IGT. Thus, treatment with 12 puffs was followed by a significant 29.6% decrease in mean plasma glucose at two-hours and a 26.8% decrease at three-hours Considering all time points OGTT, there was a mean reduction of 15.8% in mean plasma glucose following buccal spray insulin. No hypoglycaemia episodes were recorded. The aim of this study was to evaluate the effect of Oral-lyn™ on metabolic and immunological parameters in subjects affected by IGT who were exposed to long term treatment with this insulin formulation.
Material and Methods: We have designed a randomized controlled trial in 36 subjects with IGT comparing buccal spray insulin (12 puffs per meal) plus physical exercise and diet vs. physical exercise and diet only (control group). Primary endpoint is the reduction of HbA1c of 0.3 % at 6 month treatment between the experimental vs control group. Secondary endpoints include the evaluation of production of antibodies against insulin (IA), glucose variability (measured with continuous glucose monitoring), changes in body weight, number of hypoglycemic events after buccal spray insulin treatment. Insuiln antibodies were measured using a DASP recognized assay.
Results: Subjects enrolled in the treatment group did not suffer of hypoglycaemia episodes. IA were negative at entry into the study in IGT subjects and treatment with buccal spray insulin did not induce generation of IA .
Conclusion: Our preliminary data show that subjects treated with buccal spray insulin do not develop autoimmunity vs insulin as usually occurs with subcutaneous or other forms of insulin delivery (pulmonary). This may represent an additional benefit of buccal insulin, considering also the more acceptable route of administration.
Supported by: Generex

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Clinical performance of the insulin infusion set InsuPatch that applies local heat to the infusion site

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Background and aims: The importance of good glycemic control in therapy of people with type 1 diabetes is well known. Using rapid acting insulin analogues, early postprandial hyperglycemias and late postprandial hypoglycemias are still common. In this study, the effect of the local skin heating device InsuPatch on postprandial blood glucose levels after differently composed meals was investigated.
Materials and methods: Twenty-four type 1 diabetic subjects on CSII (10 male, 14 female, age: 43.5±11.3 years, diabetes duration 18.3±10.5 years, HbA1c 7.4±0.8%, daily insulin need 0.58±0.15 U/kg/d, BMI 25.0±3.0 kg/m2 [mean ± SD]) were included in this study. With data from an intensive monitoring phase of five days, the therapy parameters were optimized. During a clinical visit, subjects had standardized meals on four days, with the InsuPatch device heating on two days. Two pairs of breakfast meals (65% carbohydrates, 15% protein, 20% fat) and two pairs of dinner meals (40% carbohydrates, 20% protein, 40% fat) were obtained for evaluation. The impact of local skin heating on insulin absorption was measured using the normalized AUC 0-120 min above baseline blood glucose (BG).
Results: A total of 42 breakfast meal pairs and 38 dinner meal pairs were usable for the analysis. A significant difference between heated and not heated InsuPatch was found for the normalized AUC 0-120 min. The difference for dinner meals ($p<0.005$; AUC 0-120 for BG above baseline not heated 30.8±31.0 mg/dl, heated 18.4±23.9 mg/dl), representing slowly resorbed meals, was stronger than for breakfast meals, representing fast resorbed meals ($p<0.05$, AUC 0-120 for BG above baseline not heated 66.4±32.8 mg/dl, heated 56.8±34.0 mg/dl). The BG level above baseline 90 minutes after the meal was significantly lower with heated InsuPatch device especially after dinner meals ($p<0.001$, BG90 above baseline not heated 41.1±47.9 mg/dl, heated 25.1±37.2 mg/dl). The AUC 0-60 minutes for the venous insulin concentration after breakfast was significantly larger with heated InsuPatch ($p<0.001$, AUC 0-60 for insulin concentration above baseline not heated 42.8±23.4 mU/l, heated 53.1±28.8 mU/l). The number of hypoglycemic events showed no statistically significant difference.
Conclusion: This study shows that local heating of the skin around the infusion site was significantly increasing early insulin levels post delivery as well as significantly reducing post-prandial blood glucose increase without causing more hypoglycemia. The device provides faster insulin action, however it has to be further evaluated to which extent the device is effective in daily life use.
Supported by: Roche Diagnostics GmbH

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Biocompatibility of the ultra-rapid insulin VIAject® with continuous insulin infusion sets

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Background and aims: Use of prandial insulins with a more rapid absorption profile would be expected to provide improved postprandial glucose control with lower risk of hypoglycemia during treatment with continuous subcutaneous insulin infusion. Viaject is a formulation of recombinant human insulin that has been shown to be more rapidly absorbed than insulin lispro or regular insulin. The goal of this investigation was to explore the biocompatibility of VIAject with CSII infusion systems from several insulin pump manufacturers.
Materials and methods: Infusion sets used for the following insulin pumps were included into this investigation: (ACCUCHEK® Combo (Roche Diagnostics), Paradigm® 722 (Minimed-Medtronic), Animas® IR2020 (Animas, Johnson&Johnson), and Omnipod® (Insulet®). Three catheters (steel needle and/or Teflon catheter) of each product were tested. The pumps were filled with VIAject and emptied over 96 h at 37°C though the infusion systems. Samples of the delivered insulin product composition were collected after 0, 1, 3 and 4 days. Determinations of insulin, its degradation products and high molecular weight proteins were performed by a USP-conform HPLC-method.
Results: All mean insulin concentrations and by-product concentrations were in the acceptable ranges by USP specifications (insulin: 95-105 %, A21 desamido insulin < 2 %, total contamination excl. A21 desamido insulin < 2 %, high molecular weight products < 1.7 %). The results were similar to those of a reference sample of insulin VIAject stored in a glass vial under the same environmental conditions.
Conclusion: This investigation confirmed that VIAject is pharmacologically stable when delivered though clinically used infusion systems under the conditions of CSII therapy. The pharmacological properties of VIAject appear suitable for pump use which can now be investigated in clinical trials.
Supported by: Biodel, Inc.

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A pocket instrument for calculating insulin need in the management of type 1 diabetes

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Background: Intensive insulin therapy is today the gold standard form of therapy in patients with type 1 diabetes. For achieving optimal metabolic control, adjustments of the insulin dose at meal times must be made before each injection by taking into account several parameters including blood glucose levels, the insulin/carbohydrate ratio, the carbohydrate intake at each meal and the intensity of physical exercise post injection. A new tool recently developed for the establishment of the insulin dose to be administer (Calsulin) takes into account all above parameters in a matter of seconds and

displays the insulin units to be injected. Aim of this randomised trial was to evaluate the efficacy of Calsulin on metabolic control as assessed by HbA1c in patients with type 1 diabetes undergoing intensive insulin therapy.
Material and methods: A total of 40 consecutive patients affected by type 1 diabetes aged 18-65 years with disease duration > 1 year, were included in the study. HbA1c was evaluated at entry into the trial and at 3 and 6 months follow-up. Patients were randomised to Calsulin or standard education for insulin treatment (control group). Paired t test (two tailed) and analysis of variance were used to evaluate differences in HbA1c at different time points.
Results: HbA1c at entry was 7.9% ± 1.0 (SD) in Calsulin treated group and 7,8% ± 1.6 (SD) in control patients (p:NS). Already after 3 months follow-up there was a tendency for an improvement in HbA1c levels in the Calsulin treated group vs. control group (7.3% ± 0.5 vs. 7.7% ± 1.0, respectively, p:NS). Taking into account the 6 months period of observation, a statistically significant reduction in HbA1c levels was observed in the Calsulin treated group vs. control group (-0.85% vs. -0.07% difference, respectively, p<0.05).
Conclusions: The results of this study showed that this simple pocket instrument of the size of a small calculator is an acceptable and practical tool to make the process of calculating the number of insulin units very simple and, most importantly, helps to improve metabolic control as shown by a significant reduction of HbA1c levels compared to standard methods used for calculating the required insulin doses.

PS 90 Long-acting insulin analogues

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Insulin degludec: less pharmacodynamic variability than insulin glargine under steady state conditions

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Background and aims: Insulin degludec (IDeg) is a new generation basal insulin analogue with an ultra-long (>24-hour half-life) duration of action. We compared the pharmacodynamic (PD) variability of IDeg and insulin glargine (IGlar) under steady-state conditions.
Materials and methods: In this randomised, double-blind, parallel-group study, 54 subjects with type 1 diabetes (48 male, 6 female, age 38±10 years (mean±SD), HbA1c 7.7±0.9%, BMI 24.6±2.2 kg/m²) received 0.4 U/kg of either IDeg or IGlar once daily for 12 days. On treatment days 6, 9 and 12 PD-profiles were investigated over 24h with the euglycaemic glucose clamp technique (Biostator, pre-dose blood glucose (BG) stabilisation at the clamp level of 5.5 mmol/l with iv insulin that was stopped post-dose when BG decreased by 0.3 mmol/l and when glucose infusion was initiated). Within-subject variability (expressed as coefficient of variation - CV) was estimated using a linear mixed model on log-transformed PD endpoints. All PD endpoints were derived from the glucose infusion rate (GIR) profiles during the clamps.
Results: IDeg produced significantly less overall PD variability than IGlar between days 6, 9 and 12 on all protocol-specified PD variability parameters including total metabolic effect (AUC-$GIR_{0\text{-}24h}$, CV 20 vs. 82%, p<0.0001), the effect in the last 22 hours (AUC-$GIR_{2\text{-}24h}$ (not influenced by iv insulin during the clamp), CV 22 vs. 92%, p<0.0001) and the maximum effect (GIR_{max}, CV 18 vs. 60%, p<0.0001). Total metabolic effect (AUC-$GIR_{0\text{-}24h}$) tended to be higher with IDeg than with IGlar (geometric mean 2618 vs 1953 mg/kg, ratio 134%). The individual within-subject variability was consistently lower for IDeg compared with IGlar when the individual CVs were compared in a ranked order (figure). IDeg's metabolic effect was exactly evenly distributed between the first and the second twelve hours (ratio of AUC-$GIR_{0\text{-}12h}$/AUC-$GIR_{0\text{-}24h}$ geometric mean: 0.50 vs. 0.57 with IGlar) and this distribution was also less variable than with IGlar (CV 10 vs 17%, p<0.001). Both insulin formulations were well tolerated. No serious adverse events occurred. In total, 166 (20 nocturnal) hypoglycaemic episodes, defined as BG < 2.8 mmol/l with or without hypoglycaemic symptoms, were observed with IDeg compared with 182 (37 nocturnal) episodes with IGlar. There were no severe hypoglycaemic episodes in this study. No injection site reactions occurred with either insulin.
Conclusion: Under steady-state conditions after once daily administration, the effect of the novel basal insulin analogue insulin degludec is evenly distributed over each 24 h period and significantly less variable than that of insulin glargine. The results suggest a less variable and more stable glucose-lowering insulin effect for IDeg compared with IGlar.

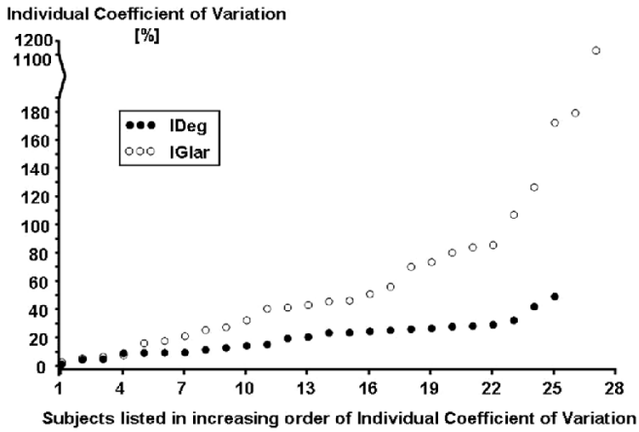

Figure: Individual within-subject variability in AUC-$GIR_{0\text{-}24h}$ for IDeg and GLA

Supported by: Novo Nordisk A/S

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Insulin degludec: multi-hexamer formation is the underlying basis for this new generation ultra-long acting basal insulin

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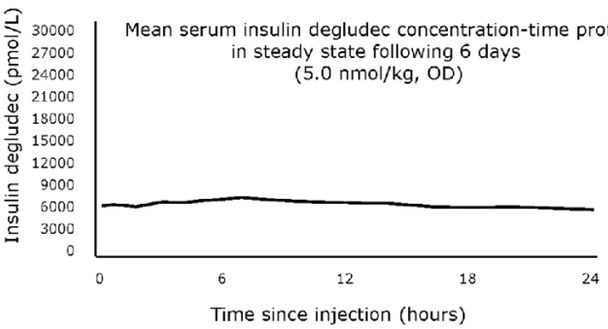
Background and aims: Insulin degludec is a new generation ultra-long acting basal insulin analogue in clinical development. The insulin degludec molecule retains the human insulin amino acid sequence except for the deletion of ThrB30 and the addition of a 16-carbon fatty diacid attached to LysB29 via a glutamic acid spacer. It is well established that absorption rate from subcutaneous tissue is determined by molecular size. Therefore the aim of this study was to demonstrate that under *in vitro* conditions mimicking the physiological injection site, insulin degludec self-associates to form large multi-hexamer assemblies and that this ultimately results in an ultra-long and peak-less pharmacokinetic profile when administered to people with type 1 diabetes.
Materials and methods: Size exclusion chromatography (SEC) experiments were performed to characterise the molecular size of self-assembled units of insulin degludec, in particular, hexamers, di-hexamers and multi-hexamers. Various pharmaceutical formulations containing zinc ions, phenol and m-cresol were examined by SEC analysis to simulate conditions before and after sub-cutaneous injection. To examine the pharmacokinetic (PK) profile of insulin degludec, a clinical pharmacology study was conducted in subjects (n=12) with type 1 diabetes. The steady state PK profile (24 hour) was determined after 6 consecutive days of once daily dosing with insulin degludec (5.0 nmol/kg).
Results: SEC analysis demonstrated that insulin degludec forms di-hexamers in the presence of phenol and m-cresol. To mimic a subcutaneous injection, further SEC analysis was conducted in the absence of phenol and m-cresol and it was revealed that there occurs a reorganisation from di-hexamers to multi-hexamer assemblies which remain in solution at physiological pH. In the clinical pharmacology study where insulin degludec was administered to subjects with type 1 diabetes, the steady-state PK profile demonstrated a smooth and stable exposure over 24 hours (see figure). Insulin degludec was found to have a t½ longer than 24 hours and was detectable in circulation for at least 96 hours after the final injection.
Conclusion: In summary, insulin degludec is a new generation soluble basal insulin with an ultra-long, peak-less pharmacokinetic profile attributed to multi-hexamer formation and slow release of insulin degludec monomers. Insulin degludec has the potential to address major challenges in diabetes care such as hypoglycaemia as well as compliance by providing more flexible dosing schedules.



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Insulin deglucec, a new generation ultra-long acting insulin, in a mealtime + basal regimen in people with type 1 diabetes: comparison to insulin glargine

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Background and aims: Insulin degludec (IDeg) is a novel insulin analogue that forms soluble multi-hexamer assemblies after subcutaneous injection (s.c.), resulting in ultra-long duration of action. This phase 2, 16-week, open-label, randomised, three-arm, parallel-group trial investigated the efficacy and safety of candidate formulations of IDeg in people with type 1 diabetes.
Materials and methods: Participants (mean age 45.8 years, HbA_{1c} 8.4 %, fasting plasma glucose (FPG) 9.9 mmol/l, BMI 26.9 kg/m^2) injected (s.c.) IDeg (n=59), an alternative formulation of IDeg (development discontinued, data not shown; n=60) or insulin glargine (IGlar; n=59) once daily in the evening, combined with meal-time insulin aspart. Basal insulin was titrated to achieve FPG 4.0-6.0 mmol/l.
Results: At 16 weeks, mean HbA_{1c} was comparable (IDeg 7.8%, IGlar 7.6%; estimated treatment difference = 0.1% (SE 0.1%)), as was FPG (IDeg 8.3 mmol/l, IGlar 8.9 mmol/l; estimated treatment difference = -0.6 mmol/l (SE 0.7 mmol/l)). At end-of-trial, mean total daily insulin dose was comparable to baseline (IDeg 60 U/day; IGlar 51 U/day), with minimal increases in mean basal insulin dose for both IDeg (from 29 to 30 U/day) and IGlar (from 23 to 26 U/day). The rate of confirmed hypoglycaemia (plasma glucose < 3.1 mmol/l or requiring assistance) was 28% lower for IDeg compared to IGlar (47.9 vs. 66.2 events/patient year; relative rate = 0.72 (95% CI: 0.52; 1.00)). The overall rate of confirmed nocturnal hypoglycaemia was 58% lower for IDeg compared with IGlar (5.1 vs. 12.3 events/patient year; relative rate = 0.42 (0.25; 0.69)). Very few severe hypoglycaemic events were reported for IDeg and IGlar (7 vs. 6 events). The overall rate of adverse events was similar between basal insulins, with no specific pattern or clustering. No injection site reactions were observed.
Conclusion: In this proof-of-concept trial, IDeg was safe and well-tolerated and provided comparable glycaemic control to IGlar at similar doses, with a reduced rate of confirmed hypoglycaemia.
Supported by: Novo Nordisk A/S

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Insulin degludec: a new ultra-long, basal insulin designed to maintain full metabolic effect while minimizing mitogenic potential

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Background and aims: Insulin degludec (IDeg) is a new generation basal insulin analogue in clinical development, designed to allow the formation of soluble multi-hexamer assemblies upon subcutaneous injection to give an ultra-long peak-less pharmacokinetic profile. The aim of the present study was to investigate the metabolic responses and molecular safety (IGF-1 receptor affinity and in vitro mitogenicity) of IDeg.
Materials and methods: Insulin and IGF-1 receptor binding studies were conducted using recombinant human insulin receptors (both isoforms, hIR-A and hIR-B) and human IGF-1 receptors. Scintillation proximity assays using solubilised receptors from transfected BHK cells were conducted in the absence of albumin. Receptor kinetic studies were conducted using intact BHK cells expressing the hIR-A. The mitogenic effect of insulin degludec was determined by measuring ^{3}H-thymidine incorporation into L6 myoblasts expressing HIRs (L6-HIR), primary human mammary epithelial cells (HMEC) as well as COLO-205 and MCF-7cell lines (from human colon and mammary adenocarcinomas, respectively). The metabolic effects of IDeg were determined by: (1) lipogenesis in rat adipocytes (^{3}H-glucose into lipid), (2) glycogen accumulation in rat hepatocytes and (3) glycogen synthesis in rat skeletal muscle cells, L6-HIR and MCF-7 cells (^{14}C-glucose into glycogen).
Results: The affinity of IDeg for both human insulin receptor isoforms (HIR-A and - B) was found to be similar (13% and 15% relative to human insulin,

HI) while the affinity for the human IGF-1 receptor was lower (2% relative to HI). The kinetics of IDeg binding to the hIRA was not significantly different from that of HI. The mitogenic response measured in L6-HIR, HMEC, COLO-205 and MCF-7 cells, in the absence of alubmin, ranged from 4-14% relative to HI. IDeg elicited the same metabolic responses and same maximal effect as HI: lipogenesis in rat adipoctes, glycogen accumulation in rat hepatocytes and glycogen synthesis in rat muscle cells. Furthermore, in cellular assays where no albumin was added (hepatocytes and MCF-7 cells), the *in vitro* metabolic potency was determined to be in the range of 8-20% resulting in a mitogenic/metabolic potency ratio of ≤1.
Conclusion: In summary, insulin degludec is a full agonist at the insulin receptor and maintains the metabolic responses of HI. This is in agreement with phase 2 trial results, where IDeg gave comparable glycaemic control as insulin glargine at similar molar and unit doses in subjects with type 1 or 2 diabetes. The low IGF-1 receptor binding affinity and the low mitogenic/metabolic potency ratio indicate that the modifications made to IDeg do not compromise molecular safety.
Supported by: Novo Nordisk A/S

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Once-daily use of a new generation ultra-long acting basal insulin with a bolus boost in insulin-naïve people with type 2 diabetes: comparison with insulin glargine

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Background and aims: Insulin degludec (IDeg) is a novel insulin analogue that forms soluble multi-hexamer assemblies after subcutaneous injection, resulting in ultra-long duration of action. IDegAsp is a soluble insulin product comprising IDeg (70%) and insulin aspart (IAsp, 30%). The aim of this phase 2, 16-week, open-label, randomised, parallel-group, treat-to-target trial was to investigate the safety and efficacy of IDegAsp in insulin-naïve people with type 2 diabetes inadequately controlled on oral antidiabetic drugs.
Materials and methods: Subjects (mean: 59.1 yrs, HbA_{1c} 8.5%, fasting plasma glucose (FPG) 11.6 mmol/l, BMI 30.3 kg/m^2) received once-daily IDegAsp (n=59), an alternative formulation of IDegAsp (AF: 55% IDeg and 45% IAsp; n=59) or insulin glargine (IGlar; n=60), all in combination with metformin, for 16 weeks. Insulin was dosed (s.c.) before dinner and titrated to a FPG target of 4.0-6.0 mmol/l. At the end of the 16-week treatment period, patients underwent a 72-h continuous glucose measurement (CGM).
Results: After 16 weeks, mean HbA_{1c} decreased from baseline in all treatment groups (IDegAsp: -1.31%; AF: -1.46%; IGlar: -1.29%) to comparable end-of-trial values (IDegAsp: 7.0%; AF: 7.2%; IGlar: 7.1%; *p*=NS for all pairwise comparisons). A similar proportion of subjects achieved HbA_{1c}<7.0% without confirmed hypoglycaemia in the last 4 weeks of treatment (IDegAsp: 51%; AF: 47%; IGlar: 50%). Mean self-measured 2-h post-dinner PG increment was lower for IDegAsp (0.13 mmol/l) and AF (0.24 mmol/l) than IGlar (1.63 mmol/l). These findings were mirrored by mean 2-h post-dinner interstitial glucose (IG) increments determined by CGM. The mean total time spent in hyperglycaemia (IG>12 mmol/l) per day tended to be lower for IDegAsp (2.2 h) and AF (2.2 h) compared to IGlar (2.7 h). Mean FPG was similar across treatments (IDegAsp: 6.8 mmol/l; AF: 7.4 mmol/l; IGlar: 7.0 mmol/l). At end-of-trial, mean daily insulin doses were lower for IDegAsp (0.38 U/kg) and AF (0.36 U/kg) than IGlar (0.45 U/kg).
No severe hypoglycaemic events were reported. Rates of confirmed hypoglycaemia (PG<3.1 mmol/l) were lower for IDegAsp and IGlar than AF (1.2, 0.7 and 2.4 events/patient year). The proportion of subjects having at least one episode of near-hypoglycaemia (IG<3.5 mmol/l) over the course of a 72-h CGM was similar amongst groups (IDegAsp: 46%; AF: 44%; IGlar: 54%). Very few confirmed nocturnal hypoglycaemic events were reported for IDegAsp (1 subject; 1 event) and IGlar (3 subjects; 3 events) compared to AF (10 subjects; 27 events). Adverse events with a possible or probable relation to insulin were only reported for AF (5 subjects; 5 events).
Conclusion: This proof-of-concept trial showed once-daily IDegAsp to be safe, well tolerated and effective. IDegAsp provided comparable overall glycemic control to IGlar at similar rates of hypoglycaemia, with the additional benefit of post-dinner PG control.
Supported by: Novo Nordisk A/S

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Efficacy and goal attainment with insulin glargine vs comparators

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Background and aims: Clinicians have many type 2 diabetes (T2DM) treatment options. We evaluated patient level data from similar prospective, randomized, controlled studies to assess the likelihood of achieving glycemic control after 24 weeks of insulin glargine (GLAR) vs comparators (C) by baseline glycated hemoglobin A1C (A1C) category (<8.0%, 8.0% to <9.0%, 9.0% to <10.0%, and ≥10.0%).
Materials and methods: Data were pooled from 9 similar studies (N=2938) meeting the following criteria: included insulin-naive patients with T2DM, uncontrolled on oral antidiabetic drugs (OADs), randomized to addition of GLAR (n=1462) or C (OADs, NPH, lispro, or premix; n=1476) and utilized similar insulin titration algorithms.
Results: Groups were comparable at baseline (56% male, 83.5% white, mean age 57 y, diabetes duration 8.6 y, A1C 8.7%, body mass index 31.6 kg/m^2). A1C reductions at week 24 were greater with GLAR vs all C (-1.68 and -1.51%, *P*<0.001); and when evaluated vs specific comparators, reductions were greater with GLAR vs OADs and vs. premixed insulin, but similar to NPH and lispro. Proportions of patients achieving ≥1.0% reduction in A1C and achieving A1C ≤7.0% were greater with GLAR vs all C, and vs OADs (Table). The comparisons vs specific insulins failed to reach significance. When stratified by baseline A1C category, A1C improvements were similar between GLAR and OADs if A1C <8.0% but greater with GLAR if A1C ≥8.0%; similar between GLAR and other insulin C for all categories except a greater reduction if <8.0% vs premixed insulin. Hypoglycemia event rates were significantly lower for GLAR vs NPH, lispro, and premixed insulin, and higher for GLAR vs OADs (Table). Severe hypoglycemia was lower for GLAR than premixed insulin (0.02 vs 0.08 events\patient\year, *P*< 0.05), and not different between GLAR and OADs, NPH, or lispro. Changes in weight were similar between GLAR and any C. Incidence of edema was higher with OADs than GLAR, largely as the result of 2 trials in which the OAD comparator was a thiazolidinedione.
Conclusion: Initiating GLAR in patients uncontrolled on OADs was associated with better efficacy and goal attainment overall, vs C across the A1C continuum, and compared with OADs when baseline A1C was ≥8.0%. Efficacy across A1C categories was similar for insulin comparators; however, hypoglycemia rates were lower with GLAR.

Table. A1C and Hypoglycemia

Treatment	Week 24 A1C ≤7.0% (%)	Hypoglycemia Event Rate (Any), Events/Patient/Year
Insulin glargine, n=1462[b]	57.7	7.13
Comparator, n=1476	51.4	10.12
Odds ratio (95% CI)	1.353 (1.161, 1.576)	
P value	<0.001	<0.001
Insulin glargine, n=460[c]	55.2	4.52
OADs, n=482	44.0	3.05
Odds ratio (95% CI)	1.69 (1.29, 2.215)	
P value	<0.001	<0.001
Insulin glargine, n=416[d]	58.7	12.25
NPH, n=429	55.9	15.37
Odds ratio (95% CI)	1.179 (0.883, 1.575)	
P value	0.264	0.032
Insulin glargine, n=198[e]	65.2	4.39
Insulin lispro, n=204	73.0	15.98
Odds ratio (95% CI)	0.685 (0.444, 1.058)	
P value	0.088	<0.001
Insulin glargine, n=287[e,f]	48.1	6.85
Premixed insulin, n=278	41.7	10.95
Odds ratio (95% CI)	1.392 (0.975, 1.988)	
P value	0.069	0.004

[a]Adjusted mean; [b]9 studies; [c]3 studies; [d]2 studies; [e]1 study; [f]OADs discontinued in premixed arm only (1 study).

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Improved glycaemic controls for patients on twice daily dosing regime of insulin glargine compared to those on a once daily dosing regime

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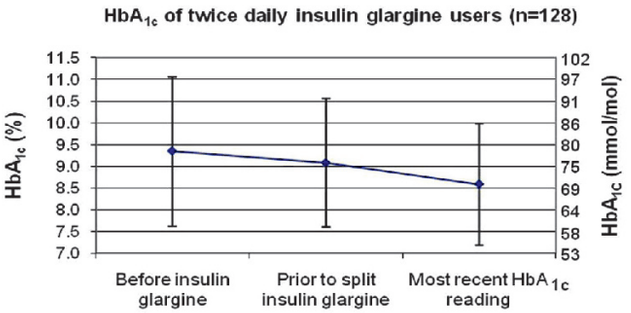
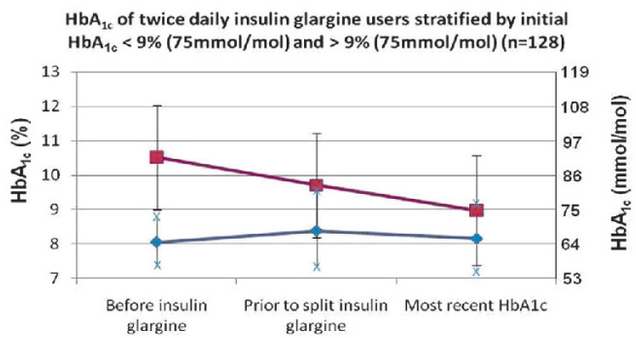
Background and aims: Insulin glargine is a long acting once-daily insulin formulation, which is used to achieve glycemic control in patients with type 1 or type 2 diabetes mellitus. It is unclear whether better glycaemic control, in terms of HbA_{1C}, is achieved with twice daily dosing of insulin glargine compared to once daily adminstration.
Materials and methods: We conducted a retrospective case note analysis to evaluate whether there is any change in HbA_{1C} values among type 1 or type 2 diabetic patients after switching from a once-daily to twice daily insulin glargine regime. Data was collected as a part of a local service evaulation on diabetes care using patient notes and database laboratory results.
Results: A total of 206 patients were included with 38% on once daily insulin glargine (n=78) and 62% on twice daily insulin glargine (n=128). Of the 128 patients using a twice daily insulin glargine dosing regime, we found that switching from previous insulin therapy to once daily insulin glargine was associated with a average decrease in HbA_{1C} of 0.27% (3 mmol.mol) and there was a further decrease of 0.49% (5.4 mmol/mol) when the insulin glargine was changed to a twice daily dosing regime. This decrease was further evident when starting HbA_{1C} was greater than 9%.
Conclusion: Our data suggests that patients on a one daily dosing regime of insulin glargine may benefit from reduction in HbA_{1C} levels by switching to a twice-daily insulin glargine regime.





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MAGE revisited: Evaluation of glucose variability with once daily glargine vs NPH insulin in type 2 diabetes

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Background and aims: Insulin combination regimens, i.e. a single bedtime injection of long-acting insulin added to oral anti-hyperglycaemic agents, are widely used in the secondary failure treatment of type 2 diabetes (t2DM); however most basal insulin formulations do not provide a constant and reliable 24-h insulin supply and are associated with an high risk of nocturnal or early morning hypoglycaemia. Aim of this perspective study was to evaluate the efficacy and safety of once daily glargine vs NPH insulin on glucose control and variability, expressed by Service's MAGE (Mean Amplitude of Glycaemic Excursions), in combination therapy of t2DM.
Materials and methods: In 40 t2DM patients, aged 62.5±8.3, overweight (BMI 29.1±6.0 kg/sq.m)and in a poor glycaemic control (HbA1c 9.1±1.2%)on a combination treatment with once daily bedtime NPH insulin 0.21±0.07 IU/kg/day and oral agents, sulphonylureas and metformin, after a 2-weeks run-in-period NPH was replaced by the same dose of glargine at the same time while oral agents were unchanged. Body weight, HbA1c and fasting C-peptide were evaluated at baseline and after 6 weeks during which, as well as in 2-weeks run-in-period, the patients provided a weekly self-monitored 6-points blood glucose profile (before and 2 h after breakfast, lunch and dinner) and recorded daily any hypoglycarmic episode. The mean glucose values of self-monitored profiles in run-in-period were compared point by point with the glargine treatment last 2 weeks profiles and MAGE was calculated.
Results: After glargine 6-weeks treatment body weight (as BMI) and C-peptide levels were unchanged; a slight but not significant decrease was observed in HbA1c (8.8±1.4 p=0.052); only fasting blood glucose (p=0.042)and 2-h post-breakfast glucose (p=0.028)significantly decreased while MAGE reached a more significant difference (71.4±15.6 vs 92.3±16.5 mg/dl p<0.001); moreover on glargine treatment the percentage of symptomatic hypoglycaemias was significantly lower (- 39.5% p=0.012).
Conclusion: Glargine insulin instead of NPH did not improve the short-term glycaemic control in combination therapy of t2DM patients:only two mean glucose levels significantly decreased in the six-points self-monitored profile while decrease of HbA1c was not significant. On the contrary glargine treatment was effective to reduce glucose variability, according to highly significant decrease of MAGE, and to lower the frequency and seriousness of hypoglycaemic episodes. In a blind post-study interview with DTSQ 29 patients (72.5%) declared an improvement of well-being, likely due to the lesser extent of glycaemic excursions.

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Efficacy and safety of insulin lispro protamine suspension versus insulin glargine added to oral antihyperglycaemic medications and exenatide in patients with type 2 diabetes

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Background and aims: Patients (pts) with type 2 diabetes (T2D) on oral anti-hyperglycaemic medications (OAMs) and exenatide (Ex) may encounter progressive metabolic deterioration requiring additional treatment. Intensification of therapy with insulin added to OAM(s) plus Ex has not been previously reported. The primary aim of this study was to determine if insulin lispro protamine suspension (ILPS) is noninferior to glargine (G) in change in HbA_{1c} when added to OAMs plus Ex in adults with suboptimally controlled T2D.
Materials and Methods: This open-label, multicenter, randomised, 24-week clinical trial enrolled pts with T2D (BMI ≤45 kg/m^2, HbA_{1c} ≥7.0% and ≤10%) who had been treated ≥3 months with 1 or 2 OAMs (metformin ± sulphonylurea or pioglitazone) and Ex (10 µg twice-daily). Pts were randomly assigned to receive either ILPS (n=171) or G (n=168) at bedtime added to pre-study OAM(s) and Ex. Insulin was titrated from 6 units daily, using dosing algorithms to achieve fasting plasma glucose (FPG) targets 4.4-5.5 mmol/l for ILPS and 4.1-5.5 mmol/l for G. Statistical analysis was performed based on intent-to-treat population using last observation carried forward method. Prespecified noninferiority margin was 0.4%.
Results: Baseline demographics (age 56 years; T2D duration 9.9 years; weight 102 kg; BMI 34.9 kg/m^2) and disease characteristics (table) were similar across treatment groups. At 24-week endpoint (table), least squares mean difference in HbA_{1c} change between treatment groups (ILPS minus G) was 0.22% (95% CI: 0.06 to 0.38), demonstrating noninferiority of ILPS to G. However, mean reduction in HbA_{1c} was statistically less for ILPS-treated patients than G-treated pts. Percentage of pts who achieved HbA_{1c} <7.0% was not significantly different between treatment groups. Endpoint FPG was similar between treatment groups. Insulin dose was lower for ILPS vs G. Overall and severe hypoglycemic rates were similar in both groups but nocturnal hypoglycaemia rate was higher in ILPS- vs G-treated pts. Weight gain, <1 kg, was similar between treatments. Serious adverse events were infrequent and similar between groups.

Conclusion: ILPS is noninferior to G for change in HbA_{1c}. Compared to G, ILPS-treated pts had higher nocturnal hypoglycaemia rates, but similarly low overall and severe hypoglycaemia and minimal weight gain over the 24-week study duration. This is the first study demonstrating that addition of ILPS or G as treat-to-target basal insulin is an effective option for improving glycaemic control in pts with suboptimally controlled T2D treated with OAM(s) and Ex.

	ILPS (n=171)	Glargine (n=168)	p-value
Baseline HbA_{1c} (%)	8.20 ± 0.77	8.23 ± 0.80	0.888
Endpoint HbA_{1c} (%)	7.04 ± 0.81	6.83 ± 0.78	0.008
HbA_{1c} change (%)	-1.16 ± 0.84	-1.40 ± 0.97	0.008
Pts achieving HbA_{1c}<7.0% (n [%])	87 (53.7%)	100 (61.7%)	0.177
Baseline FPG (mmol/l)	9.74 ± 2.19	10.04 ± 2.16	0.231
Endpoint FPG (mmol/l)	7.20 ± 1.75	7.05 ± 1.61	0.179
Insulin dose (IU)	31.1 ± 18.9	37.9 ± 18.5	<0.001
Insulin dose (IU/kg)	0.30 ± 0.17	0.37 ± 0.17	<0.001
Overall hypoglycaemia rate (episodes/pt/yr)	16.27 ± 23.19	18.05 ± 24.59	0.570
Nocturnal hypoglycaemia rate (episodes/pt/yr)	4.88 ± 8.43	3.01 ± 7.21	0.004
Severe hypoglycaemia incidence (n [%])	3 (1.8%)	0	0.249
Baseline Weight (kg)	101.6 ± 18.7	102.3 ± 19.7	0.718
Weight gain (kg)	0.27 ± 3.38	0.66 ± 3.93	0.343
Patients with ≥1 serious adverse event (n[%])	9 (5.3%)	5 (3.0%)	0.414

Values are presented at mean ± standard deviation unless otherwise noted

Supported by: Eli Lilly and Company

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Basal insulin NPH, glargine and detemir in type 2 diabetes: hepatospecificity, effects on glucose and lipid metabolism, and pancreatic islet alpha and beta cell rest: a PK-PD study

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Background and aims: To compare pharmacokinetics (PK) and pharmacodynamics (PD) of insulins NPH, glargine (Gla) and detemir (Det).
Materials and methods: 18 persons with type 2 diabetes (age 60±7 yrs, known diabetes duration 12.8±7.5 yrs, BMI 29.1±3.2 kg/m^2, A1C 7.5±0.6%, treatment insulin+OHA) (mean±SD) were studied after 1-week treatment with either NPH, Gla or Det 1/day (at 10 pm). Plasma glucose (PG) was clamped at 100 mg/dl for 32 h after s.c. injection of 0.4 U/kg at 10 pm (randomized, single-blind, crossover study). The primary endpoint was the glucose infusion rate (GIR) over 0-32 h (AUC_{0-32h}).
Results: All 18 persons completed the Gla study, but 2 persons on NPH and 3 on Det, interrupted the study because PG >150 mg/dl. The target PG was best achieved by Gla, followed by NPH and Det (102±2, 108±15, 108±14 mg/dl, p<0.05). However, PG increased overnight with NPH to a peak (119±18 mg/dl) at 07.00 h (NPH vs Gla and Det, p<0.05). The GIR AUC_{0-32h} was greater for Gla vs Det and NPH (1538±688, 1081±785, 1170±703 mg/Kg, p<0.05). Plasma insulin peaked with NPH at 05.00h and then decreased (peak 95±46, 32h 44±24 pmol/l), whereas it decreased less with with Gla (peak at 07.00h 108±42, 32h 61±30 pmol/l). Det (bound+free) had a plateau of ~1.800 pmol/l between 6-10 am followed by relatively faster decrease vs Gla. Gla suppressed endogenous glucose production more than NPH and Det (p<0.05), with no effect on glucose utilization. Glucagon was lower with Gla vs Det by 10% (p<0.05) likewise C-peptide with Gla vs Det and NPH (-17% and -12%) (p<0.05). FFA were higher with Det vs Gla by 22% and NPH by 15% (p<0.05) likewise ketones vs Gla (41%) and NPH (20%) (p<0.05).
Conclusion: All 3 basal insulins have similar hepato-specific effects, but there is no unit-to-unit equivalence on glucose-lipid metabolism. NPH is limited by nocturnal peak and decreased PK/PD at dawn. Det and Gla are superior to NPH for overnight-morning PG control, but Det is less potent than Gla and NPH in the afternoon. Each basal insulin should be used according to its PK/PD.

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Mechanism for the differential effect of the long-acting insulin analog detemir on weight in patients with type 1 diabetes

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Background: The acylated long-acting insulin analog detemir appears to lack the usual propensity for insulin to cause weight gain. Possible mechanisms include insulin detemir's predominant action on liver giving it a more physiological profile as well as a direct and indirect effect on appetite. Elucidation of the mechanism(s) of weight sparing with insulin detemir could provide valuable insights into the cause of insulin induced weight gain.
Research, design and methods: A single-centre, 32 week, open-label randomized crossover design trial was undertaken in 23 subjects (14 men, age 36.8 ±10.6 years, BMI 28.0 ±3.6 kg/m2) (mean±SD) with type 1 diabetes. Patients on a basal-bolus regime (with insulin aspart as bolus insulin) were randomized to receive either insulin detemir or NPH insulin as basal insulin for 16 weeks, followed by a switch to the other basal insulin for 16 weeks. At the end of each 16 week period the following were measured: total energy expenditure (by double labelled water), resting energy expenditure and diet induced thermogenesis (by indirect calorimetry), activity energy expenditure, (with an Actiheart monitor) and energy intake (by a 7 day food diary). Weight change, glycemic control, hypoglycemic episodes and hormones that affect satiety/fuel partitioning were also measured. Following a standard meal (600 kcals), serial measurements of GLP-1, ghrelin, pancreatic polypeptide and peptide YY were undertaken for 180 minutes. Statistical analysis was done using a general linear mixed model, and it was modified to include additionally a repeated measure effect for the times of measurement of the metabolic hormones.
Results: After 16 weeks of treatment, the weight change was -0.69 ± 1.85 kg with insulin detemir and +1.7 ± 2.46 kg with NPH (p=0.0006).Total energy expenditure was not different with insulin detemir compared to NPH insulin (p=0.334) but total energy intake was significantly less with insulin detemir (2016± 501 kcal/day) than NPH insulin (2181± 559 kcal/day) (p=0.026). There was no significant difference in HbA1c or the number of hypoglycemic episodes. Statistical modeling showed there was no relationship between HbA1c or hypoglycemia and weight change. Leptin was significantly lower with insulin detemir (9.45±7.29 ng/ml) compared to NPH insulin (10.83±9.15 ng/ml, p=0.039). Resistin was significantly higher with detemir compared to NPH insulin treatment (12.10±9.43 ng/ml vs. 9.28±4.13 ng/ml, p=0.047). There was no difference in adiponectin and IGF-1. Following the meal, ghrelin (610.9 pg/ml vs. 528.39 pg/ml, p=0.002) and pancreatic polypeptide (813.4 vs. 777.13 pg/ml, p=0.001) were significantly higher with insulin detemir compared to NPH treatment. There was no difference in GLP-1 and PYY levels.
Conclusion: Insulin detemir caused less weight gain in type 1 diabetes patients compared to NPH insulin. This study suggests this is due to reduced energy intake rather than an increase in energy expenditure. This may be mediated by a direct or indirect effect of insulin detemir on hormones that control satiety.
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PS 91 Body and soul: the psychological aspects of diabetes

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Psychological distress predicts the development of the metabolic syndrome: a prospective, population-based study

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Background and aims: To prospectively examine the association of psychological distress with the development of metabolic syndrome (MetS) and the role of potential mediators (demographic characteristics, health behaviors and inflammation) in this association.
Materials and methods: A total of 466 (185 male and 281 female) subjects, aged 36 to 56 years and free of MetS at baseline, participated in a population-based study from 1997-1998 and again from 2004-2005. Mean observation time was 6.4 years. Various clinical, biochemical and behavioral factors were measured at baseline, including assessment of psychological distress using the 12-item General Health Questionnaire (GHQ-12). The development of MetS was measured at follow-up based on National Cholesterol Education Program (NCEP) criteria.
Results: Subjects with high psychological distress at baseline (GHQ score 4-12) were more than twice as likely to develop MetS than those with low psychological distress (OR 2.18, 95% CI 1.30 to 3.64). Adjustments for age and gender, health behaviors (smoking, alcohol use and leisure time physical activity) and C-reactive protein (CRP) in the analysis diminished the odds of developing MetS in the distressed group (OR 1.87, 1.82 and 1.80, respectively); however, the association remained statistically significant (p=0.022 - 0.037).
Conclusions: Psychological distress at baseline increases the risk of developing MetS during follow-up. This association remained robust after adjusting for age, gender, baseline health behaviors and CRP. These prospective findings are evidence of a significant association between psychological distress and the development of MetS. Thus, effective treatment of psychological distress may reduce the incidence of MetS.

TABLE 1. Logistic Regression Models for the Metabolic Syndrome across Follow-up (Odds Ratios, 95% Confidence Intervals, p-values). Models Include Psychological Distress, Age, Gender, Health Behaviors and C - reactive Protein.

	Odds ratio (95% CI)	p-value
Psychological distress (GHQ-12)		
Low (0-3)	1 (reference) †	
High (4-12)	1.80 (1.04 to 3.12)	0.037
Age, per year	1.09 (1.05 to 1.14)	<0.001
Gender		
Female	1 (reference) †	
Male	1.02 (0.64 to 1.65)	0.92
Current smoker	1.77 (1.05 to 2.99)	0.032
Current use of alcohol	0.85 (0.45 to 1.62)	0.62
Leisure time physical activity		
High	1 (reference) †	
Moderate	1.25 (0.74 to 2.10)	
Low	0.95 (0.43 to 2.10)	0.85‡
hsCRP, per mg/l	1.03 (0.93 to 1.13)	0.58

Model 1 = Psychological distress.
Model 2 = Psychological distress, age, gender.
Model 3 = Psychological distress, age, gender, smoking, use of alcohol, leisure time physical activity.
Model 4 = Psychological distress, age, gender, smoking, use of alcohol, leisure time physical activity, hsCRP.
GHQ-12 = 12-item General Health Questionnaire; hsCRP = high-sensitivity C-reactive protein
Leisure time physical activity (min. 30 minutes) = Low: < 1 time/week, Medium: 1-3 times/week, High: >3 times/week
† Denominator (reference group) of following odds ratios
‡ p-value for linearity across the leisure time physical activity groups

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A matched case-control study of depressive symptoms in type 2 diabetes

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Background and aims: Diabetes and depression are important co-morbid conditions. Patients with diabetes are 1.5-2 times more likely to have depression compared with people without diabetes, although this risk is attenuated after adjustment for age, sex and cardiovascular disease (CVD). Risk factors for depression include both clinical and personal factors, and there is some evidence that psychiatric conditions including depression are more common in rural vs. urban environments. We conducted an age- and sex-matched case-control study to elucidate the relationship between type 2 diabetes and depression in a rural setting.
Materials and methods: In 2009, residents of Busselton Shire in the south-west of Western Australia who had been diagnosed with diabetes and randomly selected age- and sex-matched normoglycaemic residents were invited for a comprehensive assessment. In addition to medical history, examination and biochemical testing, participants completed the Personal Health Questionnaire Depression Scale (PHQ-9). Paired tests were used to compare potential associates of depression between cases and controls. Multiple logistic regression was used to determine independent associates of prevalent depression.
Results: We assessed 172 adults with type 2 diabetes and 172 controls. Half (51.2%) were male. Cases and controls did not differ significantly in age (70.7±10.4 vs. 71.0±10.0, P=0.80), but cases had significantly higher mean body mass index (BMI) than controls (30.4±5.3 vs. 27.0±4.0, P<0.001) and higher prevalence of self-reported CVD, exertional chest pain, and current smoking habit. Those with diabetes had median duration of 8.9 [5.0-14.3] years; 35.7% were diet-treated, 46.8% were on oral treatment and 17.5% were using insulin. Those with type 2 diabetes were significantly more likely to have a current major or any depressive syndrome compared with those with normoglycaemia (5.9% vs. 0.6%, P=0.012, and 11.8% vs. 4.1%, P=0.019, respectively), but were no more likely to have been prescribed antidepressant therapy (11.6% vs. 12.6%, P=0.86). The majority (22/25 or 88.0%) of normoglycaemic subjects with any depressive syndrome and/or taking antidepressants were being treated for depression compared with less than two-thirds (20/35 or 57.1%) of those with type 2 diabetes. BMI, current smoking habit and exertional chest pain but not diabetes status were independently associated with the presence of a) any depressive syndrome, and b) any depressive syndrome and/or antidepressant medication use. Age, sex, income, marital status, born overseas, education, alcohol intake, and self-reported CVD were also not associated with depression.
Conclusion: Depressive syndromes, especially major depression, were significantly more prevalent in rural-dwelling Australian adults with type 2 diabetes compared with age- and sex-matched normoglycaemic controls, but those with diabetes were less likely to be treated for depression. The higher prevalence of depressive syndrome in adults with type 2 diabetes compared with normoglycaemic adults may be explained largely by their significantly higher BMI.
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Depression, glycaemic control, and physical activity in a multi-ethnic population screened for type 2 diabetes

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Background and aims: Individuals with type 2 diabetes mellitus (T2DM) suffer from higher levels of diagnosed depression and depressive symptoms compared to healthy controls. Evidence also exists for this relationship in individuals with impaired glucose regulation (IGR), defined as impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). It is unclear whether this association is related to lifestyle factors such as physical activity (PA) or pathophysiological changes related to insulin resistance. Previous studies have shown an association between depression and PA levels, and individuals with T2DM and IGR are less physically active than healthy controls. We hypothesised that glucose regulation is associated with depression, independent of PA levels.

Materials and methods: Participants were identified from general practices in Leicestershire (UK), using the Leicester Diabetes Risk Score. Individuals attended a baseline screening visit that involved questionnaires, blood samples and clinical assessment. Glucose status was assessed using the Oral Glucose Tolerance Test. Depression status was assessed using the Hospital Anxiety and Depression Scale (HADS). Participants wore a pedometer for a 7 day period to objectively measure PA levels. SPSS v.16 was used to calculate means (± s.d.) and correlation coefficients. ANOVA and chi squared tests assessed differences between groups.
Results: 926 individuals (38.4%female) were screened; 88.6% White European and 11.4% Black and Minority Ethnic. Mean age was 63 years (±8.3); 25.7% had screen-detected IGR and 4.4% had screen-detected T2DM. Mild-severe depression was detected in 9.6% of screened individuals and 17.8% reported a history of depressive illness. Mean steps per day was 6801 (±3202). Mild to severe depressive symptoms were more prevalent in individuals with screen-detected T2DM (19% vs. 12% with IGR vs. 9% with normal glucose tolerance (NGT), $p < 0.05$ for trend). Across the whole sample fasting glucose was weakly correlated with depression score when controlling for age, sex and ethnic origin ($r = 0.087$, $p < 0.05$), which was maintained after further controlling for PA levels ($r = 0.083$, $p < 0.05$). In addition, fasting glucose was higher in those with moderate-severe depressive symptoms, compared to those without depressive symptoms (mean difference = 0.48mmol/l, p = < 0.05). No significant associations were found between depression score and 2-hour glucose or HbA1c. In participants with screen-detected IGR, fasting glucose was more strongly correlated with depression score when controlled for age, sex and ethnic origin ($r = 0.247$, p = < 0.01), which was maintained after further controlling for PA levels ($r = 0.269$, $p < 0.01$). Those with IGR performed significantly less steps/day than individuals with NGT (mean difference = -850 steps, $p < 0.01$).
Conclusion: This study reveals a positive association between depression score and fasting glucose, independent of PA levels, in individuals that are unaware of their glycaemic status. This suggests that the higher levels of depression seen in those with T2DM are not simply due to the impact of living with a chronic illness or to the low levels of PA seen in this group. We did not observe an association between depression score and 2-hour glucose or HbA1c, suggesting that the mechanisms underlying IFG may be more related to depression than those relating to IGT.
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Interaction between depressive symptoms and illness representations in predicting self-care behaviours in type 2 diabetic patients
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Background and aims: Predictive value of illness representations including seriousness, treatment effectiveness and control, on multiple diabetes outcomes has been well established. This study aimed to determine how depressive symptoms interacted with personal model's domains in predicting eating behaviour, physical activity, blood glucose self-monitoring and foot care in type 2 diabetic patients.
Materials and methods: A randomly selected sample of 172 type 2 outpatients (55% female, aged 63±8, educated for 10.5±4 yrs., with diabetes duration of 10±8 yrs., 29% insulin-treated, with 6.5%±0.9 HbA1C and 30±4 kg/m2 BMI) was interviewed for psychological anamnesis and examined for depressive symptoms and personal model of diabetes. Respondents' mood and illness representations were assessed by The Patient Health Questionnaire-Depression (PhQ-9) and the 8-item Personal Model of Diabetes Questionnaire (PM), and their self-care behaviours were approximated by the Summary of Diabetes Self-Care Activities (SDSCA). Participants were divided into three groups: without depressive symptoms (PhQ<5), with minimal symptoms (PhQ=5-9) and with moderate to severe depressive symptoms (PhQ≥10), which were compared as to personal model, self-reported self-care and disease-related characteristics. Kruskal-Wallis ANOVA was used to compare psychological and disease-related variables in the groups with different depressive symptom levels, and ordinal multinomial regression model to determine to what degree depressive symptoms and personal model of diabetes predicted particular self-care behaviours.
Results: Minimal depressive symptoms were reported by 20% of the patients, and moderate to severe symptoms by another 9%. No differences in age, diabetes duration, type of therapy, HbA1C and BMI were found between the non-depressed group and patients with depressive symptoms (all p>0.05). Self-reported eating behaviour, exercise and foot care were comparable across the groups (all p>0.05). Blood glucose self-monitoring was most frequent in patients with minimal depressive symptoms, followed by the non-depressed and moderately to severely depressed ones (H=5.80 p=0.05). Diabetes impact on daily life as measured by PM was reported greater by both depressed groups as compared to the non-depressed one (H=6.18 p=0.04), while other domains - seriousness, diabetes control and prevention of complications - did not differ across the groups. Non-linear regression model indicated that the control beliefs independently predicted patients' eating behaviour and physical activity as measured by SDSCA (Wald Stat=9.3 p=0.002 and Wald Stat=7.4 p=0.006, respectively). Foot care was predicted by seriousness beliefs (Wald Stat=4.6 p=0.03), while depressive symptoms were the only independent predictor of blood glucose self-monitoring (Wald Stat=7.9 p=0.01).
Conclusion: Even mild depressive symptoms were found to be associated with illness representations in type 2 diabetic patients, implying a more highly perceived impact of diabetes on daily activities. Beliefs that self care controlled diabetes were the best predictors of eating behaviour and exercise, while feelings about diabetes seriousness and worry predicted foot care. Depressive symptoms rather than personal model domains predicted blood glucose self-monitoring, showing a positive effect in mild depressive symptoms and adverse in moderate to severe ones.
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Pain self-efficacy and pain catastrophising predict depression in people with painful diabetic neuropathy
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Background and aims: Neuropathy is one of the most common and troublesome complications of diabetes and neuropathic pain can result in significant morbidity and reduced quality of life. The objective of the study was to investigate whether pain self-efficacy (belief in one's ability to function, despite pain) and pain catastrophizing (fearful and anxious thoughts about pain) influenced depression levels in those with painful diabetic neuropathy.
Materials and methods: We identified a sample of 138 patients with painful diabetic neuropathy on the basis of database recordings and issued a postal survey pack including a measure on pain (Brief Pain Inventory - BPI), pain self-efficacy (Pain Self-Efficacy Scale - PSE), pain catastrophizing (Pain Catastrophizing Scale - PCS), and depression (Hospital Anxiety and Depression Scale - HADS).
Results: We received responses from 62 patients (45% response) of mean age 54 years; 79% had Type 2 diabetes. Depression "caseness" on the HADS was reported by 55% of respondents. There was a statistically significant negative correlation between Pain Self-Efficacy and Depression (r=-.54, p≤.005) and a significant positive correlation between Pain Catastrophizing and Depression (r=.511, p≤.005). When applied as a predictive model of depression, together Self Efficacy and Catastrophizing were moderately associated with Depression (Multiple R=.56) and predicted 33% of this variable's variance. The standardised regression coefficients suggest that Self Efficacy (β=-.357) is a stronger predictor of Depression than total Pain Catastrophizing score (β=.259).
Conclusion: The results indicate that severity of depression in those with diabetic neuropathic pain is predicted by a lower sense of control over the pain (as indicated by Self Efficacy measure) and is also predicted by a tendency to think about the pain in an unhelpful way (as indicated by the Pain Catastrophizing Scale). The results highlight a role for cognitive-behavioural interventions to assist patients with diabetes in coping with neuropathic pain.

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Association of self-reported hypoglycaemia and quality of life and depression among adults with type 2 diabetes mellitus
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Background and aims: This study examined the association of hypoglycemia with quality of life and depression among adults with type 2 diabetes mellitus (T2DM) with and without hypoglycemia.

Materials and methods: Respondents to the 2008 U.S. Study to Help Improve Early evaluation and management of risk factors Leading to Diabetes (SHIELD) survey were asked the number of times they experienced hypoglycemia in the past 4 weeks and past 12 months. Respondents also completed the Short Form-12 (SF-12) quality-of-life questionnaire and the Patient Health Questionnaire (PHQ-9) depression questionnaire. T2DM respondents reporting at least 1 hypoglycemia (low blood sugar) episode were compared with T2DM respondents who did not report hypoglycemia in the previous 12 months.
Results: There were 3,000 respondents with T2DM, and 2,718 (91%) completed the SF-12 and PHQ-9; 23% reported experiencing hypoglycemia in the past 12 months. Respondents reporting at least 1 hypoglycemic episode (n = 627) had significantly lower (p<0.001) SF-12 scores for both physical health (PCS) (mean ± SD: 37.4 ± 12.7 vs. 40.9 ± 12.7) and mental health (MCS) (50.1 ± 11.7 vs. 52.4 ± 10.1) compared with those without hypoglycemia (n = 2,091). Mean PCS scores decreased as the number of hypoglycemia episodes increased: PCS 39.0± 14.4 for 1 episode, 38.3 ± 12.0 for 2-3 episodes, 38.1 ± 13.3 for 4-5 episodes, and 35.1 ± 12.6 for ≥6 episodes (p = 0.03). Mean MCS scores did not differ by across these same groupings (p = 0.09). Mean PHQ-9 scores were significantly higher (p <0.001) among respondents reporting hypoglycemia (5.2 ± 5.8), compared with respondents who did not report hypoglycemia (3.9 ± 5.0), indicating greater depression burden. Significantly more respondents experiencing hypoglycemia (10.1% ± 3.8%) reported moderately severe to severe depression (PHQ-9 scores ≥15), compared with respondents without hypoglycemia (5.1% ± 2.1%). Mean PHQ-9 scores increased as the number of hypoglycemia episodes increased: score 4.1 ± 4.9 for 1 episode, 4.9 ± 5.7 for 2-3 episodes, 5.7 ± 6.3 for 4-5 episodes, and 6.0 ± 6.0 for ≥ 6 episodes (p = 0.01).
Conclusion: T2DM respondents experiencing hypoglycemia report a lower quality of life, in the domains of both physical and mental health, and greater burden of depression than respondents without hypoglycemia. These findings suggest that hypoglycemia and depression need to be considered together in routine clinical practice settings.
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Can affects and self image affect diabetes mellitus?
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Background and aims: Depression is common in patients with DM and associated with impaired metabolic control and increased risk of all diabetic complications. Other types of psychological and psychosomatic factors involved in DM morbidity are not so well investigated. Our aims in this study were to explore depression, anxiety, alexithymia and self image and their correlations with HbA1c among young and middle aged patients with DM. Alexithymia was here defined by three factors: Difficulties Identifying Feelings (DIF), Difficulties Describing Feelings (DDF) and Externally Oriented Thinking (EOT). Self image was in this study composed of three positive factors: "self-affirm", "active self-love", "self-protect"; three negative factors: "self-blame", "self-attack", "self-neglect"; and two neutral factors reflecting autonomy: "self-emancipate" and "self-control".
Materials and methods: At a Swedish specialist outpatient clinic, 353 DM patients 18-59 years old (median age 42 years; 56% men, 44% women) participated in this study. Depression and anxiety were assessed by the Hospital Anxiety and Depression Scale (HAD), Alexithymia by the Toronto Alexithymia Scale-20 (TAS-20), and Structural Analysis of Social Behaviour (SASB) was used for assessing the eight factors of self image described above. We also measured waist circumference and determined Body Mass Index (BMI) for the 353 DM patients. We did bivariate correlation, linear regression, and combined variable correlations analyses with HbA1c as dependant variable.
Results: Mean HbA1C was 7.13 % (SD=1.37, N=353) and mean waist circumference 0.87 m (SD 0.13, N=339). An elevated anxiety score was found in 35% (111/320), a negative self image in 22% (69/309), alexithymia in 16% (51/318), and an elevated depression score in 12% (37/320) of the 353 DM patients. We found that 45% (N=144) of DM patients did not have any signs of depression, anxiety or alexithymia and had a normal self image score. Their mean HbA1c was 6.96 % (SD=1.18). Alexithymia had the greatest impact (p=0.037) and the combination with negative self image (p=0.035) was particularly severe. For 17 patients with a high alexithymia score, a negative self image, but with a normal depression score HbA1c was 7.82 % (SD=1.57). For 12 patients with a combination of a high alexithymia score, a negative self image and a high depression score HbA1c was 8.57 % (SD=1.78). For 46 women with a waist circumference of > 0.88 m the HbA1c was 7.92 %, and for 21 men with >1.02 it was 7.35%. Bivariate correlation between HbA1c and 17 variables showed a significant r below the 5% p-level for the following 8 variables: anxiety, depression, DIF, "self-control", "self-attack", "self-neglect", BMI, and waist circumference. Age, DDF, EOT, "self-emancipate", "self-affirm", "active self-love", "self- protect", "self-blame" and duration of DM showed no correlation with HbA1c. In linear regression analyses, including the 8 variables above, DIF (p<0.0001) and waist circumference (p<0.001) remained significantly associated with HbA1c.
Conclusion: In this study we show that alexithymia and negative self image had a greater impact on HbA1c levels than depression in patients with DM. Particularly, the alexithymia sub factor DIF (difficulties identifying feelings) correlated with HbA1c, and the waist circumference in a linear regression model.
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Longitudinal motivational predictors of dietary self-care, life satisfaction and diabetes control in adults with newly diagnosed type 2 diabetes mellitus
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Background and aims: Changing dietary habits is considered the most difficult part of diabetes self-management after being diagnosed with type 2 diabetes (T2DM). The tendency to overeat at diagnosis has been associated with weight gain and intake of energy four years after diagnosis. Therefore, better understanding of the motivational factors involved in adopting and maintaining dietary self-care activities during the critical period following the diagnosis of T2DM is needed. This study aimed to examine whether motivational factors from Social-Cognitive Theory (self-efficacy, positive and negative outcome expectancies and self-evaluation) and Self-Determination Theory (autonomous self-regulation and autonomy support) are associated with the course of dietary self-care, life satisfaction and diabetes control in a sample of people newly diagnosed with type 2 diabetes.
Materials and methods: Participants, 237 people (65% male) newly diagnosed with type 2 diabetes, completed questionnaires assessing perceptions of support from health care professionals fostering autonomy, autonomous motivation, dietary self-efficacy, positive and negative outcome expectancies, self-evaluation, dietary self-care, life-satisfaction and diabetes knowledge at five occasions, each three months apart. BMI and HbA1c were also assessed on each occasion.
Results: Multivariate analyses, using Generalised Estimating Equations (GEE), showed that autonomous motivation ($\beta = 0.06$, $p < 0.05$), dietary self-efficacy ($\beta = 0.006$, $p < 0.001$), and self evaluation ($\beta = 0.23$, $p < 0.001$) were significantly associated with the course of dietary self-care, even when adjusted for demographic and illness-related variables. The course of life satisfaction over the 18-month period was significantly associated with self-efficacy ($\beta = 0.008$, $p < 0.01$), and positive ($\beta = 0.005$, $p < 0.01$) and negative outcome expectancies ($\beta = -0.002$, $p < 0.01$), while changes in HbA1c were significantly associated with self-efficacy ($\beta = -0.007$, $p < 0.05$), and negative outcome expectancies ($\beta = -0.005$, $p < 0.01$). However, for the latter two analyses, self-efficacy was no longer a significant predictor after controlling for a number of demographic and illness-related variables.
Conclusion: Together with self-efficacy and autonomous motivation, self-evaluation is an important motivational construct associated with dietary self-care over the first 18 months after diagnosis of T2DM. Whether people newly diagnosed with T2DM remain satisfied with their lives over that period depends on their positive and negative perceptions they have about their diabetes. Because HbA1c reflects diabetes control over the past 2-3 months, the significant longitudinal association with negative outcome expectancies is likely to indicate poorer control of diabetes leading to more negative perceptions of diabetes. In teaching how to cope with newly diagnosed T2DM, health care professionals may want to include specific interventions targeting these motivational constructs.
Supported by: Diabetes-UK

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The association of screening anxiety with fasting and 2-hour plasma glucose, and HbA_{1c}

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Background and aims: Previous work has identified that feelings of stress can result in hyperglycaemia, which may become problematic during diabetes screening involving Oral Glucose Tolerance Tests (OGTTs). OGTTs are currently the 'gold standard' approach for screening for diabetes, and involve collection of both fasting and 2-hour plasma samples. Some research has identified that for diabetes screening, anxiety may be intensified in people with negative perceptions about the condition being life-threatening and resulting in complications. Importantly, research has not yet explored the extent to which raised anxiety levels can affect glucose results obtained from OGTTs. HbA1C has recently been suggested for use as a diagnostic test for diabetes. The aim of this study was to investigate associations between anxiety and fasting and 2-hour plasma glucose levels collected from OGTTs, and HbA1C.
Materials and methods: In a community screening study, 4688 White-European (WE, 40-75 years) and 1353 South-Asian participants (SA, 25-75 years) without a previous diagnosis of Type 2 Diabetes Mellitus (T2DM) underwent an OGTT, HbA1C, full lipid profile, detailed history, anthropometric measurements and completed the short-form Spielberger State Trait Anxiety Inventory. Data was analysed with means and standard deviations (for continuous variables) and percentages (for categorical variables). Pearson's correlation coefficient was computed to identify relationships. Linear modelling was conducted to further explore associations, with adjustment for confounding factors.
Results: Overall prevalence of T2DM and Impaired Glucose Regulation (IGR) was 3% and 16%, respectively. Anxiety levels were significantly higher in SA (mean 34.1; SD 0.37) compared to WE participants (mean 29.8; SD 0.13). Fasting glucose levels (mean 5.3mmol/l; SD 0.9, p=0.001) and HbA1C (mean 5.9%; SD 0.62, p<0.001) were also significantly higher among SA participants. Significant correlations were not identified between fasting (r -0.005, p=0.75) or 2-hour glucose levels (r -0.10, p=0.24) and HbA1C (r 0.01, p=0.40). Statistically non-significant associations of anxiety with fasting glucose and HbA1C remained following adjustment for age, gender or ethnicity.
Conclusion: This study found that anxiety levels at screening were heightened among people of SA ethnicity. In addition, the study found that fasting and 2-hour plasma glucose levels and HbA1C are not affected by anxiety during screening tests for diabetes. Therefore, current and proposed screening methods are not affected by anxiety at screening.
Supported by: DoH

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Patient-centered outcomes and glycaemic variability in type 1 and type 2 diabetes: a cross-over trial of insulin glargine + glulisine vs premix analogue insulin

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Background and aims: Intensive insulin therapy with multiple daily injections (MDI) offers superior glycemic control; however, regimen burden and hypoglycemia remain barriers to acceptance. The primary objective of this study was to test for superiority in improvements from baseline in patient-centered outcomes of patient satisfaction (PS) and quality of life (QoL) in subjects with type 1 or type 2 diabetes when treated with insulin glargine plus premeal rapid acting insulin glulisine versus treatment with premix analogue insulin. The secondary objectives were to compare glycemic control and variability between the two insulin regimens.
Materials and methods: We studied 388 insulin-treated patients (82 T1DM, 306 T2DM, 47% male, age 54±11 yrs, HbA1c 7.8±0.7%) who were randomized to either open-label daily insulin glargine plus premeal glulisine (GG; n=192) or BID premix 75/25 or 70/30 (PM; n=196) for 12 wks (P1), and then crossed over to the alternate treatment arm for an additional 12 wks of treatment (P2). Patients followed an insulin titration algorithm with a target HbA1c < 7.0% with the aid of an electronic diary transmitting data daily to a web-based remote monitoring system. Clinic personnel reviewed daily 4-point glucose readings, insulin dosages, hypoglycemia, other symptoms, and adverse events, and called the patient weekly to provide insulin dosing recommendations. Patients completed clinic-based PS and QoL questionnaires at Wks 0, 8, 12, 20 and 24, and underwent continuous glucose monitoring (CGM) for three-day periods at Wks 0, 12 and 24.
Results: Mean±SE HbA1c change for GG vs PM was -0.53±0.10% vs -0.20±0.10% for P1, and -0.25±0.10% vs +0.10±0.10% for P2 (both p<0.0001). At P1 Wk 12, 55% of GG reached HbA1c <7.0% vs 31% for PM (p<0.0001), with no differences in serious adverse events (5.4 vs 4.9%, p=0.7) or daytime or nocturnal hypoglycemia. Combined linear, mixed model P1 and P2 baseline-adjusted Wk 12 mean±SE estimates are reported for PS, QoL and CGM. The PS Net Benefit scale (0-100) improved from 51.1 to 60.5±1.2 for GG, but worsened to 45.4±1.2 for PM (p<0.0001). Overall QoL favored GG by 0.13±0.04 Z-score units (p<0.001). All 4 Net Benefit subscales favored GG (p<0.0001). The PS Regimen Acceptance scale was comparable (67.3±0.5 for GG vs 66.5.±0.5 for PM, p=0.33) with 3 lifestyle and side effects subscales favoring GG (p<0.001) and 3 convenience subscales favoring PM (p<0.02). QoL scales favoring GG vs PM were perceived health, symptom distress (both p<0.0001), general health perceptions (p<0.01) and psychosocial (p<0.02). Emotional and cognitive scales were comparable. CGMS daily mean, daily SD and % time > 7.8 mmol/l were lower for GG than PM by 0.7±0.1 mmol/l, 0.3±0.07 mmol/l and 7.3±1.6% respectively (all p< 0.0001), with no difference in CGM % time < 3.9 mmol/l (p=0.10).
Conclusion: Patient perception of net benefit and treatment satisfaction was more heavily weighted by the beneficial changes in health status and quality of life associated with improvements in glycemic control and reduced variability with insulin glargine plus premeal insulin glulisine than by the burden of additional daily insulin injections.
Supported by: sanofi-aventis

PS 92 The heterogeneity of diabetes

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Clinical heterogeneity of type 1 diabetes mellitus at onset

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Background and aims: Retrospective analysis was performed in 489 consecutive patients with discharge diagnosis of type 1 diabetes (T1DM) between 1999 and 2009 in our hospital to examine the clinical heterogeneity of the disease.
Materials and methods: A total of 205 out of 489 patients were newly onset T1DM. In these patients, clinical characteristics and laboratory data at onset were compared among fulminant type 1 diabetes (FT1DM, diagnostic criteria according to Imagawa et al.) and those with duration of symptoms before diagnosis shorter (acute-onset) or longer (slow-onset) than 3 months. One-way ANOVA and multivariate chi square were employed to compare the differences among groups.
Results: The proportions of FT1DM (n=18), acute-onset (n=137) and slow-onset (n=50) T1DM were 8.8%, 66.8%, 24.4% respectively. The onset of FT-1DM was so abrupt that the concentration of plasma glucose was prominently elevated, whereas $HbA1_C$ was disproportionately near normal, and the duration of symptoms before diagnosis was extremely shorter than the other two groups. More female patients tended to develop FT1DM, and flu-like symptoms (44.4% vs 22.6%, 18%) were more frequently observed in this group, but the differences failed to reach significance. Two patients who developed T1DM during or after pregnancy belonged to the FT1DM group. Ketoacidosis was almost inevitable phenomenon at diagnosis and the accompanied metabolic derangement (hyponatremia, acidosis, dysfunction of kidney and liver etc) was more severe in FT1DM group. The acute-onset T1DM constituted the maximum ratio of the disease, and patients were with the youngest age at onset and with the leanest somalotype before diagnosis. Patients with slow-onset type were relatively older and had greater body mass index but lost more weight at diagnosis. The fasting as well as post-load C-peptides in this group were relatively higher, and ketoacidosis at onset was less likely.
Conclusion: Clinical heterogeneity in the three groups was apparent, which might indicate different trigger mechanisms, especially the impacts of virus infection, feminine hormones or state of pregnancy on the extent of β cell damage as well as the development of T1DM, more so the fulminant type.
Table 1 Comparisons of clinical features of FT1DM, acute-onset and slow-onset T1DM.

Table 1 Clinical features of FT1DM, acute-onset and slow-onset T1DM.

Items	Fulminant	Acute-onset	Slow-onset	P value
Number and percentage	18 (8.8%)	137 (66.8%)	50 (24.4%)	-
Age at onset (y)	27.0±10.5	19.6±13.6	27.1±14.6	0.000*
Male sex (%)	33.3	56.2	50	0.171
Duration of symptoms	3.4±2.5 d	3.4±2.7 wk	5.7±2.7 m	0.000*
BMI before onset (kg/m^2)	20.1±3.1	18.8±4.4	20.6±3.1	0.041*
Weight loss (kg)	0.6±1.4	5.2±4.9	7.7±4.7	0.000*
Pregnancy (%) [§]	22.2% (2/9)	0	0	0.002*
Flu-like symptoms (%)	44.4	22.6	18	0.093
Ketosis at diagnosis (%)	100	88.3	84	0.071
Acidosis at diagnosis (%)	93.8	45.3	8	0.000*
Plasma Glucose (mmol/L)	31.4±11.7	25.1±10.1	24.2±8.3	0.023*
HbA1c (%)	6.8±1.1	12.3±2.4	13.9±2.7	0.000*
Na (mmol/L)	133.81±7.5	138.1±5.8	136.6±4.2	0.009*
CO2CP(mmol/L)	12.6±7.5	21.1±6.8	23.6±5.9	0.000*
Cr (μmol/L)	103.4±79.0	60.4±45.4	60.6±23.5	0.006*
AST (u/L)	55.4±60.9	39.3±46.4	34.7±51.2	0.014*
Fasting C-peptide (pmol/mL)	0.14±0.09	0.19±0.11	0.23±0.15	0.464
Post-load C-peptide (pmol/mL)	0.10±0.13	0.34±0.26	0.40±0.36	0.013*

Data are presented by the means ± SD or number of patients or percentage (%).
[§] Indicates percentage and number of female subjects aged 13–49 years who developed type 1 diabetes during or after pregnancy.
* Statistically significant difference among groups (P < 0.05).

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Ketosis prone diabetes in south London - are we discontinuing insulin appropriately?

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Background and aims: Ketosis prone diabetes is a common, but frequently unrecognised presentation of diabetes in an African/Caribbean population. An ideal approach to insulin discontinuation in these patients is unclear. We examined rates of insulin discontinuation, initial management and subsequent follow up in a large cohort of patients from a south London population.
Materials and methods: Patients that presented with diabetic ketoacidosis (DKA) or unprovoked ketosis (defined as ≥2+ ketones on urine dipstick) between 2003 and 2009 to University Hospital Lewisham, London were identified from hospital notes and a clinic database. Those with known or a clear clinical new diagnosis of type 1 diabetes were excluded.
Results: 53 patients were identified; 28 with DKA and 27 with unprovoked ketosis. 36 (68%) were male. The median age was 41 (range 17-71). Mean±SEM body mass index was 30.1±1.3. The majority were African-Caribbean in origin (76%), with 8(15%) Caucasian patients and 5(9%) from the Indian subcontinent. Thirty-seven (70%) patients presented as a new diagnosis of diabetes. Forty-six (86%) patients were discharged on subcutaneous insulin. Twenty-eight (53%) had islet cell and GAD antibodies measured; only 2 patients had positive antibodies. Sixteen (30%) patients had fasting c-peptides measured; only 1 patient had a value <330pmol/l (value used to define adequate insulin secretory capacity in a previous large cohort). The mean follow up was 31±8.4 months (range 3- 120) and 39 (74%) were still on insulin. Five patients taking insulin experienced hypoglycaemia in the first three months, leading to insulin discontinuation in 2 cases. The mean time to insulin discontinuation was 13.1±3.0 months. Only one patient had a relapse of DKA seven years after discontinuing insulin. Mean HbA1c on admission was 12.3±0.6, decreasing significantly to 8.1±0.4 at follow-up (p<0.01). Body weight did not significantly increase over follow up (90.1±3.1 admission to 91.8±3.2 follow up p=0.66).
Conclusion: This is the first large cohort of ketosis prone diabetic patients described in a UK population. Our data show that the majority of patients are discharged on insulin and continue with insulin at follow up, which may explain the extremely low incidence of relapse in DKA .Patients have a significant decrease in HbA1c with minimal weight gain, suggesting a conservative approach to insulin withdrawal may be appropriate in this population.

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Post-transplant diabetes mellitus and its impact on survival of liver transplant patients

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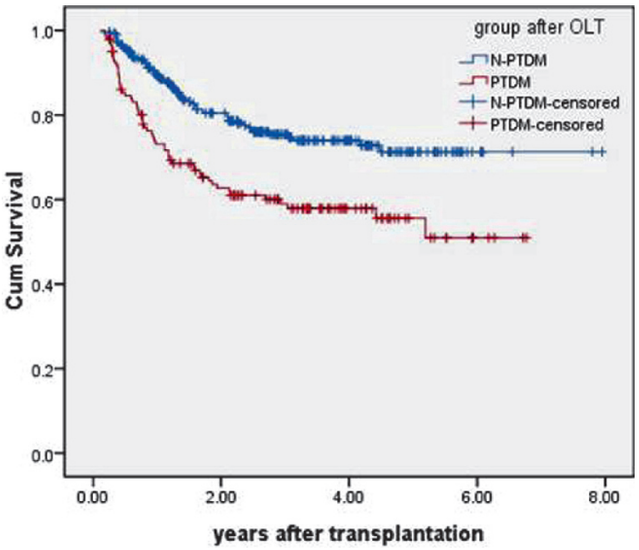
Background and aims: Post-transplant diabetes mellitus (PTDM) has great effects on complications in relation to cardiovascular system, infections, neuropsychiatric system et al, resulting in greater morbidity and affecting graft survival and patients' long-term outcome. The aim of our study was to discuss the impact of PTDM on patient survival and long-term complications.
Materials and methods: Retrospective analysis 438 patients who performed orthotopic liver transplantation (OLT) in our center between April, 2001 and December 2008, patients with previous history of using steroids, or data not completed or died within 3 months after OLT were excluded. All patients were divided to PTDM (n=140) and non-PTDM (n=298) group according to fasting plasma glucose (FPG) after operation. We compared two groups on survival rate and complications after OLT, including sepsis, chronic renal insufficiency, fungal infection, biliary complication, CMV infection, fatty liver. Cox regression analysis was used to analyze those factors affecting patient survival. We used Chi-square test to compare qualitative variables and Kaplan-Meier method and log-rank test to analyze survival rate. P value less than 0.05 was considered statistically significant. Statistic analysis was done by SPSS 16.0.
Results: Cox regression analysis indicated that FPG before or after operation, tumor relapse or metastasis and renal insufficiency after OLT were independent risk factors for patients' death, while the hazard ratio of each factor was

1.57 (P=0.02), 1.84 (P<0.0001), 2.08 (P=0.0001) in PTDM group towards non-PTDM group. The mean survival time in PTDM group was 4.216±0.260 years, while non-PTDM group was 6.133±0.198 years, which showed statistic meaning between each group (P<0.01). Compared with non-PTDM group, PTDM group had higher rate in sepsis (151/298:87/140) (P=0.025) and chronic renal insufficiency (65/298:51/140) (P=0.001), and showed statistic meaning, while no difference were found in fungal infection, biliary complication, CMV infection and fatty liver (All P>0.05).
Conclusion: PTDM has great effect on patient's survival and complication after OLT, reduces patient's survival and raises the odds of sepsis and chronic renal insufficiency.



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Patients with long-standing type 2 diabetes can develop absolute insulin deficiency

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Background and aims: Insulin treatment is increasing in Type 2 diabetes, reflecting the aim for tight glycaemic control, progressive beta-cell failure, and prolonged life expectancy. It is unclear whether the progressive beta-cell failure found in T2D can result in absolute insulin deficiency, with the resulting risk of increased fluctuations in glucose, including severe hypoglycaemia and diabetic ketoacidosis. This may need different treatment from the majority of patients with T2D who have endogenous insulin production. Recent work has developed Urinary C-Peptide Creatinine Ratio (UCPCR) as a non-invasive, stable measure of endogenous insulin production utilising a single urine sample. This has been shown to correlate well with the gold-standard Mixed Meal Tolerance Test in insulin-treated patients. We aimed to assess if absolute insulin deficiency, measured by UCPCR, occurs in T2D.
Materials and methods: We studied 171 insulin-treated subjects who clinically met criteria for type 2 diabetes (diagnosed ≥ 45 years (median age 73, IQR 67-78), and who started insulin ≥12 months post-diagnosis). They provided a spot 2hr post-prandial urine sample, on which UCPCR was measured. Absolute insulin deficiency is defined by UCPCR ≤0.2nmol/mmol.
Results: 23/171 (13.5%) had absolute insulin deficiency (UCPCR≤0.2). Duration of diabetes was significantly longer in those with insulin deficiency (18vs12yrs, p=0.02), and insulin dose in units/kg/24hrs was higher (0.77vs0.5, p=0.01). There was no difference between those with insulin deficiency versus those with endogenous insulin production (UCPCR>0.2) for age of diagnosis (median 58vs58yrs, p= 0.27), BMI (29vs29, p=0.87), HbA1c (8vs7.8, p=0.76), time to insulin from diagnosis (6vs5.5yrs, p=0.31), or number taking oral hypoglycaemic agents (OHA) (12/23 vs 92/148, p=0.36). Of those with absolute insulin deficiency, only 4/23 (17%) were on a basal bolus treatment regime, and 8/23 (34.8%) were on long-acting insulin alone.
Conclusion: UCPCR suggested 13.5% of elderly diabetic patients who met clinical criteria for type 2 diabetes had absolute insulin deficiency. Those who were insulin deficient had had a diagnosis of diabetes for significantly longer than those who retained endogenous insulin secretion, and were on higher doses of insulin. The insulin treatment regimes in the majority of these patients suggested insulin deficiency had not been recognised. Identifying insulin deficiency in long-standing patients with diabetes is important as their management will differ, and UCPCR may have a valuable role in detecting these patients.

PS 93 Tools for diagnosing and monitoring of diabetes

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How should HbA_{1c} be incorporated into the diagnostic pathway for diabetes mellitus?

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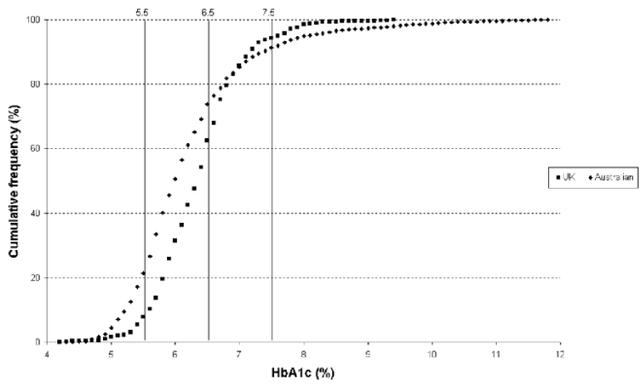
Background and aims: Alternative strategies have been suggested for using HbA_{1c} to diagnose diabetes mellitus. An HbA_{1c} cut-point of ≥6.5% has been included by the American Diabetes Association (ADA) in their guidelines for 2010. Other approaches involve limits for HbA_{1c} to 'rule diabetes out/in' and a combination of HbA_{1c} and glucose to reduce oral glucose tolerance testing (OGTT). These strategies are examined in patients referred for OGTT in the UK with impaired fasting glucose (IFG) and patients at risk of diabetes in Australia, 26% with IFG at OGTT.
Materials and methods: OGTT were performed in 500 UK patients by capillary sampling with HbA_{1c} measured by Tosoh G7 & G8 IE HPLC (ion exchange high performance liquid chromatography) analysers and in 1175 Australian patients using venous samples with HbA_{1c} from Bio-Rad Variant II Turbo analysers.
Results: The prevalence of diabetes by WHO criteria was 49% in UK patients, age, median IQ range, 62(53-72) years/52% male, and 35% for Australian patients, age 59(49-68) years/54% male. Those identified with diabetes by WHO in UK cohort were aged 62(53-73) years/52% male and by ADA 64(54-73) years/50% male, p = 0.12 for age & p = 0.54 for gender, and for Australian patients, 61(51-71) years/55% and 59(50-70) years/57%, p = 0.004 & p = 0.056. When limits of <5.5% HbA_{1c} (***N*HbA$_{1c}$** *not diabetic*) and ≥7.5% (***D*HbA$_{1c}$** *diabetic*) are applied to 'rule out/in diabetes', 12% (6%/6% respectively) of UK and 27% (17%/10%) of Australian patients would be identified. Those with ***N*HbA$_{1c}$** in the UK cohort were aged 56(46-65) years/63% male, ***I*HbA$_{1c}$** (*intermediate* 5.5% to 7.4%) 62(53-72) years/51% male and ***D*HbA$_{1c}$** 68(53-74) years/52% male, p=0.26 for age and p=0.42 for gender. For Australian patients, values were 50(39-60) years/53%, 60(52-69) years/54% and 55(47-69) years/57%, p <0.001 & p=0.48. An algorithm combining FPG <7.0mmol/l and HbA_{1c} <6.0% could provide additional benefit by reducing OGTT further as 20% of UK and 28% of Australian patients had HbA_{1c} between 5.5% and 5.9%.
Conclusion: Further studies are required to establish the sensitivity and specificity of HbA_{1c} limits in other populations. The means of referral, age and ethnicity of populations and analyser used for HbA_{1c} may influence limits chosen. In addition, use of a surrogate marker for diagnosis of diabetes will require careful assessment in individual patients of conditions, either clinical or iatrogenic, which could affect haemoglobin or its turnover.



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Diagnosis of diabetes applying the new and old ADA guidelines - characterisation of patients who do not hit both criteria

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Background and aims: For the diagnosis of diabetes the new ADA guidelines for the diagnosis of diabetes recommended the use of the HbA1c test to diagnose diabetes, with a threshold of >=6.5%. Moreover the common criteria for the diagnosis of diabetes, FPG >=7.0mmol/l and 2-h PG >=11,1mmol/l, stay valid. Not in every case the new and the old criterion lead to a diagnosis at the same time. There are patients whose glycaemic status is categorized differently either by the HbA1c or the 2 hour post challenge plasma glucose. These patients have to get characterized, to identify, which criteria is better to use in this situation.
Patients and methods: Data of 729 Patients who had 864 oral glucose tolerance tests and an HbA1c test in proximity of time were analyzed. Patients characteristics: age 40.3 y; HbA1c according to oGTT 5.5%; BMI 29.2 kg/m². HbA1c was DCCT adjusted. The conformity of diabetes-diagnosis of diabetes following the different criteria and potential reasons for non conformance were checked in the digital patient record EMIL.
Results: 28 (3.8%) of 729 patients hit both criteria for the diagnosis of diabetes (HbA1c >=6.5% and 2-h PG >=11.1 mmol/l). 641 patients (87.9%) met none of both criteria and 22 patients (3.0%) met the HbA1c (>=6.5%)- but not the glucose criterion (2-h PG >11.1mmol/l). Lots of these patients, hitting only the HbA1c criteria, had an IFG (n=16), or an IGT (n=16) or both (n=14). Four of these 22 patients had whether IFG nor IGT. In 12 patients (1.6% of all patients) with a follow up the diagnosis could be confirmed by abnormal 2h-PG at a later time. In 4 patients HbA1c dropped below 6.5%, stayed or increased and 6 patients had no or only short follow up. 5.2% (n=38) of all patients had abnormal 2-h PG (>=11.1 mmol/l) at diagnosis but HbA1c below the limit (<6.5%). In 15 patients (2.1% of all patients) with follow up HbA1c rose above 6.5% and they met both criteria for diagnosis of diabetes. Nine patients with follow up (1.9%) stayed below an HbA1c 6.5%. Fourteen 14 patients (1.9%) had no or only short follow up to show changes in HbA1c. Confounder which could interfere with Hba1c were rare (severe illness 3, anaemia 2, hypoglycaemic agents 0, corticoids 0).
Conclusion: Applying the new ADA criteria for diabetes diagnose to an caucasian population we found concordance between HbA1c criterion and 2h post challenge criterion in in 91.7% of the all patients and an additional 3.7% within 12 month after the first test. Thus, HbA1c is a suitable parameter for diagnosis of diabetes mellitus.

Conformity of HbA1c and 2h post challange plasma glucose for diagnosis of diabetes

	2-h PG <11,1mmol/l	2-h PG >=11,1mmol/l
Hba1c < 6.5%	641 patients (87.9%)	38 patients (5.2%)
Hba1c >= 6.5%	22 patients 3.0%)	28 patients (3.8%)

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Detecting undiagnosed diabetes using HbA_{1c}, an automated screening test in hospitalised patients

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Background and aims: Diabetes is associated with morbidity, mortality and use of health care resources. The prevalence of diabetes in hospitalised patients is approximately three times community prevalence. HbA1c has been recently recommended as a diagnostic tool for diabetes. The aims of this study were to assess the utility of A1C as an automated screening test for undiagnosed diabetes in hospitalised patients and to estimate the prevalence of undiagnosed diabetes in hospitalised patients.
Materials and methods: A 3 month prospective observational study of all adult patients admitted to a tertiary hospital. An A1C test was automatically undertaken on admission for all patients with random plasma glucose (RPG)

≥5.5mmol/L. Demographic, admission and biochemical data were obtained from hospital databases. A subset of patients were recruited for an oral glucose tolerance test (OGTT) post discharge. Undiagnosed diabetes was defined as A1C ≥6.5% in accordance with International Expert Committee and American Diabetes Association recommendations.

Results: The prevalence of undiagnosed diabetes was 11% (95%CI 9.8-12.4) (262/2360) during the 3 month study (figure 1). A further 312 patients with known diabetes were admitted. The prevalence of undiagnosed diabetes was highest in the 65-74 age group. In this study, the A1C test cost was US$152 per new diagnosis of diabetes. Conservatively assuming an annual incidence of undiagnosed diabetes of 0.8% the ongoing cost of testing hospitalised patients using A1C would be US$1,900 per new diagnosis of diabetes. RPG was not sensitive or specific in diagnosing diabetes. Patients were poorly compliant with OGTT, with a 27% completion rate.

Conclusion: Undiagnosed diabetes is common in hospitalised patients. A1C is a simple, inexpensive screening test that can be automated using existing clinical blood samples. Hospital screening for diabetes needs to be coupled with resources for follow up and management in the community.

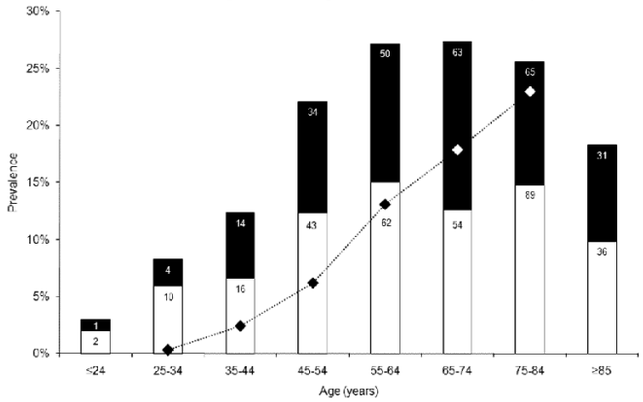

Figure 1: Prevalence (%) of diabetes in hospitalised patients according to age. The number in each group is shown within the columns. White bars indicate patients with previously known diabetes, black bars indicate patients with undiagnosed diabetes. Diamonds indicate the Australian community population prevalence.

Supported by: General Practice Education and Training

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Challenging the "Monnier Concept": High basal (not postprandial) glucose dominates hyperglycaemic exposure over a wide range of HbA_{1c} on oral therapy and contributes significantly even after addition of basal insulin

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Background and aims: Monnier et al reported that there was more contribution to overall hyperglycemia by postprandial hyperglycemia (PPHG) and less from basal hyperglycemia (BHG) at lower vs higher glycated hemoglobin A1C (A1C) levels. That analysis of 4-point profiles, from 290 patients treated with diet ± oral agents without insulin, is often interpreted as supporting the treatment of PPHG rather than BHG, unless the A1C is quite high. The current analysis was designed to study these effects of basal insulin in a larger population with more detailed glucose profiles.

Materials and methods: We evaluated data from a large population of patients with type 2 diabetes and 7-point self-measured plasma-referenced glucose profiles pooled from 6 studies in which basal insulin alone (insulin glargine or NPH insulin) was added to diet ± oral agents and was systematically titrated.

Results: 1699 participants (aged 59 ± 9 y, type 2 diabetes duration 9 ± 6 y) had baseline laboratory-measured fasting plasma glucose (FPG) of 10.7 ± 2.7 mmol/L and A1C of 8.7% ± 0.9%. After 24 to 28 weeks of insulin treatment, FPG was 6.94 ± 2.1 mmol/L, A1C was 7.0% ± 0.9%, and symptomatic hypoglycemia occurred in 60.9% of patients. We computed the contributions of BHG and PPHG to total hyperglycemic exposure above 5.55 mmol/L. Results for ranges of A1C at baseline and after treatment are shown below.

	Baseline					On Basal Insulin				
	<8.0	8.0-8.4	8.5-8.9	9.0-9.4	≥9.5	<6.5	6.5-6.9	7.0-7.4	7.5-7.9	≥8.0
N	422	348	298	245	386	437	449	351	237	225
A1C, %	7.6	8.2	8.7	9.2	10.0	6.0	6.7	7.2	7.7	8.7
BHG, %	76	78	79	79	80	42	41	42	43	48
PPHG, %	24	22	21	21	20	58	59	58	57	52

A1C correlated weakly with hyperglycemic exposure from BHG vs PPHG at baseline (P=0.074) and more significantly after improvements on basal insulin (P=0.0155). Insulin therapy reduced the basal contribution from 76%-80% to 41%-48%.

Conclusion: 1) When oral therapy is unsuccessful, BHG dominates hyperglycemic exposure over a wide range of A1C. 2) Adding basal insulin reduces basal hyperglycemic exposure and A1C, increasing the relative contribution of PPG, independent of A1C ranges. 3) BHG contributes >40% to hyperglycemic exposure, and A1C may be lowered further with basal insulin even when A1C approaches 7.0%.

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1, 5-Anhydroglucitol as a marker of short term glucose variability in well-controlled type 2 diabetes mellitus

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Background and aims: 1, 5-Anhydroglucitol (AG) is a glucose analogue present in the blood and its levels decrease when there is glycosuric hyperglycemia. The usefulness of 1, 5-AG in reflecting glycemic excursions have been demonstrated in moderately controlled patients to some extent, although some studies reveal controversial results. However, even patients with well-controlled diabetes, demonstrated by HbA1C<7%, may be subject to glycemic excursions and postprandial hyperglycemia. The aim of this study was to evaluate the role of 1,5-AG in patients with well-controlled type 2 diabetes in monitoring short term glycemic control and glucose variability, as assessed by the continuous glucose monitoring system (CGMS), when compared to fructosamine (FA).

Materials and methods: 33 patients with type 2 diabetes with HbA1C <7% with stable glycemic control were recruited. CGMS was applied to the patients for two consecutive 72-h periods. A standardized and objective approach to measure glycemic variability by CGMS_the absolute group of signs (GOS) method, which has a high correlation with the mean amplitude of glucose excursion (MAGE), was calculated. This, along with the mean postmeal maximum glucose (MPMG) and area under the curve for glucose above 180 mg/dL (AUC180), which all reflect the daily glycemic status, were compared with 1,5-AG and FA at baseline, day 4, and day 7.

Results: Baseline characteristics of the enrolled subjects were as follows: age, 56 ± 9.6 yrs; BMI, 25.5 ± 3.6 kg/m^2; DM duration, 4.9±5.0 yrs; HbA1C, 6.3±0.3%; basal 1,5-AG, 15.8±7.6 ug/mL; basal fructosamine, 276.1±23.5 umol/L. Mean 1,5-AG levels were negatively correlated with MPMG (r = -0.287, p < 0.05), AUC180 (r = -0.264, p < 0.05) and absolute GOS (r = -0.253, p < 0.05), whereas FA levels were correlated positively with fasting plasma glucose (r = 0.519, p < 0.01), MPMG (r = 0.498, p< 0.01), AUC180 (r = 0.52, p < 0.01), and absolute GOS (r = 0.285, p < 0.05). When 1,5-AG levels were divided into two groups using 14ug/mL as the cut-off value for well controlled DM, there was a statistically significant difference: 1,5-AG<14ug/mL group, compared to the 1,5-AG>14ug/mL group, had higher fasting plasma glucose (120.84±15.46mg/dL vs 109.24±11.13mg/dL, p=.002), MPMG (201.84±34.75mg/dL vs 173.13±24.41mg/dL, p=.001), AUC180 (5.10±5.49 mg/dL/day vs 1.56±2.27mg/dL/day, p=.003), and absolute GOS values (67.05±25.62 mg/dL vs 54.54 ±20.52mg/dL , p=.035), meaning greater glycemic variability.

Conclusion: 1, 5-AG reflects glycemic variability and postprandial hyperglycemia even in well-controlled DM patients, which suggests the usefulness of this as a complementary marker along with fructosamine.

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Defining the phenotype of the fast and slow deglycators by analysis of the glycation gap

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Background and aims: Despite the reliability of the Haemoglobin A_{1c} (HbA_{1c}) assays discordance between HbA_{1c} and other measures of glycaemia is often encountered. It is now recognised that there are enzyme dependent processes in the red blood cells that cause deglycation and thus influence the HbA_{1c} independent of the prevailing glycaemia. Thus the HbA_{1c} is the net of the glycation and deglycation. The rates of intracellular deglycation may vary between individuals. Fructosamine is the glycation of albumin which is extracellular and so is not affected by the intracellular deglycating enzymes. Co-analysis of HbA_{1c} and Fructosamine pairs, utilising the Fructosamine to determine a predicted HbA_{1c}, can define the 'glycation gap' (G-gap). Our objective is to determine if the glycation gap in any individual is consistent over time.
Materials and methods: We analysed all HbA_{1c} estimations (n=111205) done over a 4 year period at New Cross Hospital. 4724 people had simultaneous HbA_{1c}-Fructosamine estimations. 2263 people had at least two paired HbA_{1c}-Fructosamine estimations separated by 10±8 months. The G-gap was calculated as the HbA_{1c} minus the standardised Fructosamine derived HbA_{1c} equivalent ($FHbA_{1c}$). A negative G-gap may denote a fast deglycation state with the HbA_{1c} appearing to read lower than predicted, and a positive G-gap denotes a slow deglycation state. To ascertain the consistency of the G-gap, the G-gap for the 2nd HbA_{1c}-Fructosamine pair was calculated expecting a consistency of distribution of negative through positive values. If so, the multiple of G-gap1 and G-gap2 (whether negative or positive) will always be positive if consistent but negative with any discordance.
Results: Of the 2263 people with at least two paired HbA_{1c}-Fructosamine, their characteristics were age 60±14 years, males 55%, HbA_{1c} 8.3±1.7 (4.0-17.7) % (mean±SD (range)) and Fructosamine 308±77 (143-978) µmol/l. The $FHbA_{1c}$ was 8.3±1.7 (4.6 - 23.4) % and the HbA_{1c} minus $FHbA_{1c}$ was 0.0±1.2 (-8.2 to +5.9). Setting G-gap cut offs at <= -1, >-1 to <+1, and >= +1 as Fast, Neutral and Slow deglycators the cohort's distribution was 421 (19%), 1448 (64%) and 394 (17%) respectively. The G-gap multiple (multiple of G-gap1 and G-gap2) was 1.2±2.6 (-6.1 to +40.9). The G-gap consistency was 97% in 421 Fast deglycators (G-gap <= -1) and 99% in 394 Slow deglycators (G-gap >= +1).
Conclusion: The G-gap is consistent over time, thus by inference an individual's rates of deglycation appear to be consistent and thus this may be a method of defining the phenotypic expression of underlying metabolic and genetic processes.

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Association between grip strength and glycaemic control or diabetic complications in Korean patients with type 2 diabetes

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Background and aims: Age-related decline of muscle strength is closely related with a loss of muscle mass and an increase in fat, which are the important features in insulin-resistant states. However, very little data are available on the association of muscle strength with diabetic complications in patients with type 2 diabetes. The aim of this study was to investigate whether grip strength is associated with glycemic control and status of diabetic complication in patients with type 2 diabetes.
Materials and methods: This was an observational study performed in 193 type 2 diabetic patients with duration of diabetes within 3 years and age- and sex-matched 40 healthy individuals. Grip strength was measured by isometric dynamometry. Thigh circumference was measured for each subject. Diabetic complications were ascertained via review of medical records. Chronic kidney disease was defined as estimated glomerular filtration rate < 60 mL/min/1.73 m^2.
Results: Grip strength was correlated with age (r=-0.491, P<0.001), body mass index (r=0.133, P<0.05), and diabetes duration (r=-0.184, P<0.001). Diabetic patients had lower grip strength than healthy individuals. Especially, grip strength of poorly glycemic controlled patients (HbA1c > 7.5%) had lower than that of well controlled patients (HbA1c ≤ 7.5%). And grip strength was lower in diabetic patients with retinopathy, neuropathy, chronic kidney disease, or cardiovascular disease, compared to those without these complications. With the exception of retinopathy, these trends remained significant after adjusting for age, body mass index, waist, diabetes duration, and medications.
Conclusion: Our findings suggest that grip strength is associated with glycemic control and diabetic complications. The potential for grip strength to be used in the clinical practice of diabetic patients needs to be explored.

PS 94 Insulin pumps: a promise of improvement in metabolic control

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HbA_{1c} and sensor use in adults during a 1-year randomised controlled trial comparing sensor-augmented pump therapy and multiple daily injection therapy

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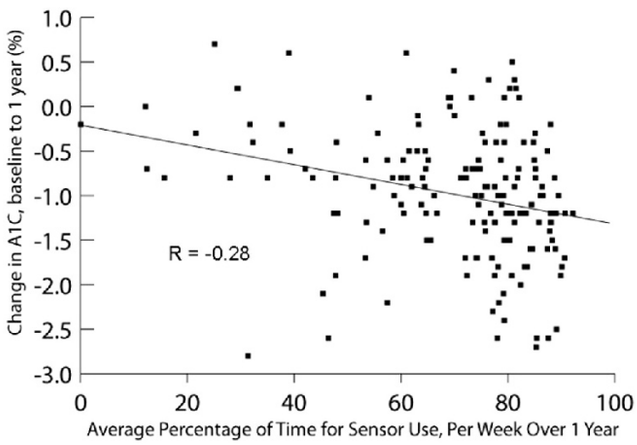
Background and aims: Recent studies have shown that continuous glucose monitoring (CGM) can improve glycemic control. The purpose of the STAR 3 study was to evaluate whether CGM combined with insulin pump therapy (sensor augmented pump, SAP) might improve A1C without increasing hypoglycemia.
Materials and methods: STAR 3 was a 1-year multicenter randomized controlled trial comparing SAP therapy to glargine- and aspart-based multiple daily injection (MDI) therapy in 329 adult (age 19-70 years) and 156 pediatric (age 7-18 years) subjects with type 1 diabetes. Preliminary results for the adult cohort of the following outcomes are given for overall A1C, A1C by age group, sensor use, and severe hypoglycemia.
Results: The primary endpoint of change in A1C from baseline to 1 year showed the decline in mean A1C levels was greater with SAP therapy compared to MDI therapy (SAP: from 8.3±0.5% to 7.3±0.7%; MDI: from 8.3±0.5% to 7.9±0.9%; treatment difference -0.6%; 95% CI, -0.77, -0.45; p<0.001). Severe hypoglycemia did not differ between treatment cohorts (p=0.53). There was a decrease in A1C from baseline to 1 year for all SAP adults of -0.9; for those 19-35 years (n=59), the decrease was -0.7; for those 36-50 years (n=58), the decrease was -1.1; and for those 51-70 years (n=49), it was -1.1. Treatment differences between groups in change in A1C significantly favored SAP therapy for all adults (-0.6, p<0.001) and in 2 age cohorts (-0.6 among subjects 19-35 years, p=0.01; -0.8 among subjects 36-50 years, p<0.001; with a favorable downward trend of -0.3 among subjects 51-70 years, p=0.16). Most SAP subjects used the glucose sensor ≥60% of the time (57.6% among subjects 19-35 years of age, 82.8% among subjects 36-50 years of age, and 91.8% among subjects 51-70 years of age). The Figure shows the r value of -.28 between sensor use and change in A1C from baseline to 1 year.
Conclusion: SAP therapy reduces A1C in adults of all ages with type 1 diabetes. Reduction in A1C appears to be related to sensor use, which showed a tendency to increase with age, but was relatively high across all ages.



Supported by: Medtronic, Inc

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Improved glucose control with sensor-augmented pump therapy in youth with type 1 diabetes and elevated HbA_{1c} levels on multiple daily injection therapy in the STAR 3 Study

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Background and aims: The effectiveness of continuous glucose monitoring (CGM) in children with type 1 diabetes (T1D) maintained on pre-existing insulin pump or multiple daily injection (MDI) treatment remains unproven. No previous study has examined the effect of initiating both CGM and insulin pump therapy simultaneously in a large pediatric cohort with T1D.
Materials and methods: This 1-year multicenter, randomized controlled trial compared an integrated sensor-augmented pump (SAP) system to MDI therapy in 156 youths with T1D with sub optimal control (A1C =7.4 - 9 .5%). A1C values were measured every 3 months and the primary outcome was the change in A1C from baseline to 12 months in the total cohort. Results were also stratified according to age (82 children, age 7-12 and 74 adolescents, age 13-18) and by the amount of time glucose sensors were worn in the SAP group.
Results: Baseline A1C levels did not differ between the SAP group (8.26±0.55%) and the MDI group (8.30±0.53%). At the end of 12 months, the change in A1C from baseline in the SAP group was -0.39±93% versus +0.16±0.95% in the MDI group, a between-groups difference of -0.49% (95% CI, -0.75 to -0.18; P=.002). The proportion of subjects reaching ISPAD-recommended A1C values (i.e., <7.5%) was 29.5% in the SAP group versus 10.3% in the MDI group (P=0.0075). As shown in the Figure, the change in A1C at 12 months in 7-12 yr old subjects favored the SAP group by -0.44% (95% CI, -0.80 to -0.07; P=0.02 vs MDI subjects) and by -0.62% (95% CI, -1.09 to -0.15, P= 0.01) in 13-18 year olds. In the entire SAP cohort, greater decrements in A1C were associated with increasing sensor wear (r=-0.30, P=0.008). Rates of severe hypoglycemia (<10 events/100 pt yrs) and DKA were low and did not differ between the two treatment groups.
Conclusion: SAP is an effective means of improving metabolic control and in achieving target A1C levels without increasing the frequency of acute complications in youth with T1D who have elevated A1C levels on MDI therapy. Increasing sensor use tends to improve the effectiveness of the SAP system.

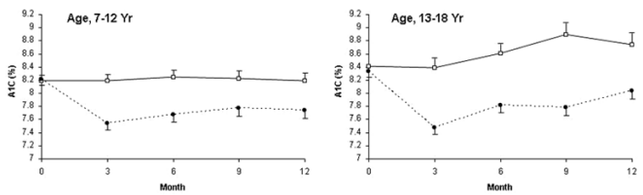

Figure. Quarterly mean (±SEM) A1C levels in pediatric and adolescent subjects. *Open squares*, MDI treatment arm; *filled circles*; SAP treatment arm.

Supported by: Medtronic, Inc.

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Long term outcomes of intensive treatment with subcutaneous insulin infusion (CSII) in patients with type 1 diabetes mellitus

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Introduction: Several studies have shown that treatment with insulin pump is an effective therapy for selected type1diabetes. Nevertheless, most of them analyse results in short period of time. The objective of this study is to evaluate long term effects on quality of life (DQOL) and metabolic control of CSII.
Material and methods: We did a longitudinal study of type 1 diabetic patients previously treated with multiple daily insulin (MDI) therapy who initiated insulin pump and maintained it for longer than three years. Initially we recruited 105 patients and after we excluded pregnant and patients using

combined sensor-pump systems. Finally we analysed in 69 patients: diabetes duration, complications, cause of initiating CSII, total daily insulin requirements, hypoglycaemias, Hba1c, DQOL, physical evaluation, number of controls and number of medical visits.
Results: The criteria for csii initiation were: 21,9% hypoglycaemic events, 37,5% poor metabolic control and 40,6% glycemic lability. After three years, 79,64% continue using insulin pump, 12,2% dropped out and 8,16% withdrawn following medical reasons.

*P<0,05	Basal	6 month	12 month	24 month	36 month
HbA1c	8.11±2,2	7.6±1,3*	7,8±0.9*	7,2±2,6*	8,2 ±0.97
Severe Hypoglycemic events/year	0,43	0,06	0	0	0,03
Hypoglycemic events/ week	4,5±3	3,3±2,8	2,1±1,7*	2,8±2,2*	2,7±1,6
Ketoacidosis with hospitalary admission	0	0	0	0	0
Sensibility factor	37,6 ±17.8	45,3±19.04	40.7±18.9	38,8±18,4	41,3±10,3
CHRatio	1.05±0,5	0,88±0,44	0.95±0.46	0.97±0,44	1,03±0,29
Autoanalysis/day	3,6±2.5	3,1±1,99	3,8±1,9	3,7±2,2	3,2±1,4
Visits / month	-	0,62±0,22	0,28±0,14	0,30±0,11	0,30±0,11
DQOL	92± 16,15	80,82± 19,2*	82,12±19,1*	82,5±14,0*	82,5±14,0*

[Long term evaluation of CSII]

Conclusions: CSII achieve a good metabolic control during the first two years and an improvement in DQOL that is maintained during all the evaluated period of time.

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Insulin requirements, basal-bolus distribution and basal patterns used by type 1 diabetic patients (T1DM) starting insulin pump therapy (CSII) and after glucose control optimisation

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Introduction: Insulin requirements of insulin at the beginning of CSII therapy is usually calculated from total daily doses (TDD) used with multiple daily injections (MDI). Basal-bolus distribution of 50%-50% with only one basal rate per day is the usual pattern at that time. However, there are only few evidences about the best form to start CSII in MDI treated patients.
Aim: The aim of the study was to assess insulin requirements, basal-bolus distribution and basal rate pattern used by a group of T1DM subjects using CSII after glucose optimization.
Patients and methods: 50 T1DM patients who started CSII therapy at 2008 were included (10 males, 40 women; 39.6±11 years; diabetes duration 19.9±11 years; HbA1c 7.9±0.9%). Indications: suboptimal glucose control (n=31) and pre-gestational optimization (n=19). TDD was calculated reducing around 20% the previous TDD in MDI regimen. TDD was divided into 50% basal (proportionally distributed along the 24 hours) and 50% pre-meal boluses (distributed according to carbohydrates at each meal). Doses and pattern distribution were modified according to glucose profile during the follow-up (1, 2 and 3-5 months after CSII initiation). After glucose profile optimization (4-6 months), TDD, basal-bolus distribution and number of basal rates were recorded and HbA1c was measured.
Results: In comparison to TDD in MDI, TDD at the beginning of CSII was reduced by 18.3%. At the end of the study the reduction of TDD was only 15.8% (non-significant). Basal rate was initially reduced by 17% and at the end by 8.6% ($p<0.05$). Basal rate represented 52.3 ± 11% of TDD at the beginning, however at the end of the study represented 58.5 ± 18% ($p<0.05$). Women needed more proportion of basal rate than males (60.6 ± 18 vs. 49.1 ± 13%; $p<0.05$), and those patients with diabetes duration >15 years, compared to those with <15 years of duration (61.8 ± 20 vs. 51.9 ± 12; $p<0.05$). Final number of basal rates/day were 5 ± 3 and this number was higher in patients with HbA1c<6.5% compared to those with HbA1c >6.5% at the end of the study (7.8 ± 4 vs. 4.1 ± 2; $p<0.05$). Basal rate infusion range was 0.81-1.01 units/hour (maximum 5-8 h AM, minimum 9-12 h AM). No differences were found between reduction in boluses at the beginning or at the end of the study for each meal. The distribution of the doses dedicated to boluses was 37% for lunch, 33% for dinner and 30% for breakfast). HbA1c levels decreased significantly 4-6 months after CSII initiation (HbA1c 7.1±0.9%; $p <0.05$).
Conclusion: In our T1DM patients starting CSII, the reduction of TDD from TDD used in MDI should be around 15%. Basal rate should be >50% of TDD especially in women and patients with longer diabetes duration (around 60%). Basal profile should be divided in > 3 patterns/day, because higher number of basal rates/day was related with better glucose control. HbA1c decreased by 0.8% 4-6 months after CSII initiation.
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Good metabolic control with a continuous subcutaneous insulin infusion (CSII) in type 2 diabetic patients uncontrolled by insulin optimisation

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Background and aims: Metabolic control in type 2 diabetic patients (T2D) treated with a continuous subcutaneous insulin infusion (CSII) is a real challenge. Moreover, it is a real great difficulty to reach in T2D the classic targets of GMC defined by normalization of Hba1c associated with no weight increasing and no severe hypoglycemia, so what is the efficacy of ambulatory insulin pump in this T2D population?
Materials and methods: A longitudinal observational study is performed since 2009, involving 28 French hospital centres in order to described circumstances of insulin pump initiation and patient management over 3 years. We have selected a population of T2D patients with a poor metabolic control despite optimization of insulin treatment (3 or 4 injections/day).
Results: To date, 161 patients have been included in the study. Follow-up data are available for 60 patients. 5 patients have stopped CSII (patient's wish - n=3, psychological reasons - n=2), and 55 patients were followed since one year: mean age 58±10 years old, 49% male and mean BMI 34±6 kg/m². 27 patients (54%) have lost at least 10% of HbA1c since their inclusion and 19 patients didn't gain over 2 kg per point of HbA1c losing between inclusion and 1 year follow-up. A Good Metabolic Control (GMC) group has been defined *a posteriori* with the following criteria: patient with no hypoglycaemia reported since inclusion who didn't gain over 2 kg per point of HbA1c losing between inclusion and 1 year follow-up and who have lost at least 10% of HbA1c since their inclusion. According to those criteria, 16 patients (30%) in our cohort are in GMC. Despite the low number of patients, comparisons on principal criteria are presented in the table below: significant differences have been logically observed on weight and HbA1c criteria. A significant higher level of HbA1c at inclusion seems to be a predictive factor for targeting GMC criteria at 1 year follow-up (p=0.07).

Comparisons on principal criteria : GMC group versus Others group

	**responding patients*	GMC group	Others group	*Test value*
Age (years)	T0	62±6	57±11	ns
Sex (% women/ % men)	T0	56/44	49/51	ns
Weight (kg)	T0	92±16	96±20	ns
	T12	93±17	99±21	ns
	Δ* T0/T12	+0.37±3.08	+4.36±5.9	0.005
IMC (kg/m²)	T0	33.15±3.77	34.41±6.50	ns
	T12	33.72±3.83	35.51±6.49	ns
	Δ* T0/T12	+0.23±1.27	+1.47±3.84	ns
HbA1c (%)	T0	9,99±2,34	8,78±1,51	0.07
	T12	7.75±1.52	8.04±1.37	ns
	Δ* T0/T12	-2.24±1.65	-0.62±1.58	0.0004
Severe hypoglycaemia (%)	Before T0	6	5	ns
	Since T0	0	0	ns
CSII associated with metformin (%)	T0	63	41	ns
Insulin dose (unit/kg/ day)	T0	0.76±0.3	0.83±0.32	ns
	Δ* Before T0/T0	-0.35±0.55	-0.23±0.37	ns
	Δ* Before T0/T12	-0.34±0.49	-0.10±0.43	ns

Conclusion: After 1 year of CSII, half of our patients obtain a good glucose control, and a third reach the targets of good metabolic control. These preliminary data emphasize the benefit of this treatment for T2D patients with poor metabolic control despite basal bolus optimization. At this stage, it is

difficult to show predictive factors of targeting GMC criteria, but further data on the global cohort could be conclusive.
Supported by: DinnoSanté

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Insulin pump therapy safely improved glycaemic control and patient reported outcomes in patients with type 2 diabetes suboptimally controlled with multiple daily injections

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Background and aims: Insulin pump therapy is an important treatment option for patients with type 2 diabetes (T2DM) who are suboptimally controlled with multiple daily injections (MDI). Limited data exist about pump therapy in this patient population. The objective of the present sub-analysis of a larger study was to assess the efficacy, safety and patient reported outcomes (PRO) of 16 wks of pump therapy in patients with T2DM suboptimally controlled with MDI therapy.
Materials and methods: In this 16-wk, open-label, multicenter study, 21 insulin pump naïve patients treated with MDI ± oral antidiabetic agents (9 male, 12 female, anti-GAD antibody negative, age 57±13, DM duration 15±6y, A1C 8.4±1.0%, FPG 9.2±3.2mmol/l, body weight 98±20kg, BMI 34±5kg/m^2, total daily insulin dose 99±65U [1.0U/kg], mean±SD) discontinued all diabetes medications except metformin and initiated insulin pump therapy (Animas® 2020 insulin pump with insulin glulisine) with one daily basal rate and bolus doses at each meal. Insulin doses were titrated to safely achieve the best possible glycemic control. The primary outcome was assessment of insulin dose and insulin dosing patterns at Wk 16. Secondary outcomes included change in A1C, fasting and postprandial glucose, body weight, PRO (Insulin Delivery System Rating Questionnaire), and hypoglycemia.
Results: Glycemic control improved significantly after 16 wks of pump therapy: A1C 7.3±1.0% (-1.1±1.2%, p<0.001) and FPG 7.2±2.1mmol/l (-2.0±4.1mmol/l, p<0.001). In patients with baseline A1C >8.5% (n=11, mean baseline A1C 9.1±0.5%), A1C was reduced by 1.9±1.3% (p<0.005). Mild hypoglycemia was experienced by 81% of patients at least once during the 16-wk study with no episodes of severe hypoglycemia. At Wk 16, the mean daily basal, bolus, and total insulin doses were 66±36U, 56±40U, and 122±72U (1.2U/kg), respectively. 90% of patients were treated with ≤2 basal rates per day (1 basal rate 80%; 2 basal rates 10%). Body weight increased by 2.7±2.6kg (p<0.001). PRO measures improved significantly from baseline (Treatment satisfaction: 65±15 vs 81±15, p<0.001; Overall treatment preference: 58±14 vs 93±16, p<0.001; Scale of 0-100, Mean±SD).
Conclusion: Insulin pump therapy using a simple dosing regimen significantly improved glycemic control in patients with T2DM who were suboptimally controlled with MDI therapy. Patients experienced moderate weight gain, no severe hypoglycemia and preferred pump therapy to baseline treatment with insulin injections. Efforts to develop simple, cost-effective insulin pumps for patients with T2DM must continue. Future controlled trials are needed to further assess the benefits of insulin pump therapy in T2DM.
Supported by: Animas Corporation

1009

A head-to-head-to-head comparison of three bolus calculators in subjects with type 1 diabetes

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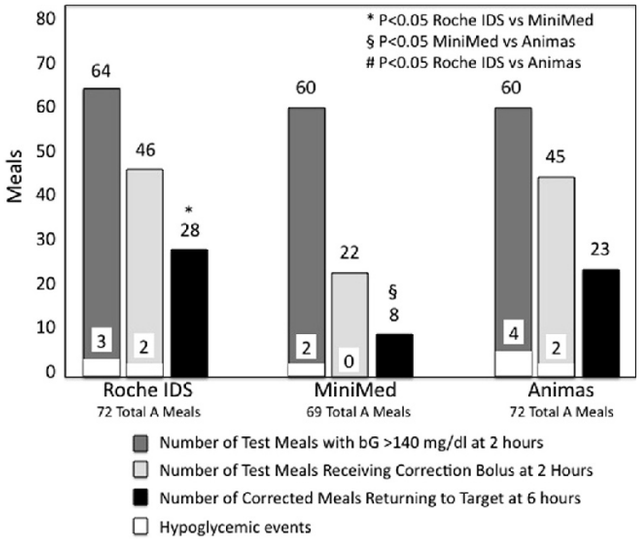
Background: Insulin pump systems now provide automated bolus calculators (ABCs) that electronically calculate insulin boluses to address carbohydrate (CHO) intake and out-of-range blood glucose (bG) levels. We compared the efficacy of three ABCs (ACCU-CHEK® Combo [Roche IDS] / Animas® 2020 [Animas] / MiniMed Paradigm Bolus Wizard® [MiniMed]) to safely reduce postprandial hyperglycemia (PPH) in type 1 diabetes mellitus (T1DM).
Methods: T1DM subjects (n=24) were recruited at a single center for a prospective, triple cross-over study. ABCs with programmed target range (80-140 mg/dl) were used in random order. PPH was induced by reducing the calculated bolus by 25%. At two hours after test meals, the ABCs were allowed to determine whether a correction bolus was needed. Differences between bG values at six hours after test meals that achieved 2-hour PPH and the mean of the target range (110 mg/dl) were determined.
Results: The mean difference between 6-h bG levels following test meals and the 110 mg/dl bG target with the MiniMed device (47.4 ±31.8 mg/dl) was significantly (P<0.05) higher than the Animas (17.3 ±30.9 mg/dl) and Roche (18.8 ±33.8 mg/dl) devices. The number of meals with 2-hour PPH and the bG levels at 2 hours was similar. Roche and Animas devices recommended correction boluses significantly more frequently than the MiniMed device (P<0.05). Significant hypoglycemia was not associated with ABC use.
Conclusion: In this study, the Roche and Animas devices were more efficacious in controlling PPH than the MiniMed device. Use of ABCs can assist in controlling postprandial glycemia without significant hypoglycemia.



Supported by: Roche

1010

Effects of evening meals with complex nutrient content on the nocturnal blood glucose levels of type 1 diabetes patients

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Background and aims: Currently no therapy recommendations are available as to how postprandial blood glucose levels can be improved after meals rich in protein and fat. Recent epidemiological studies reported reduced insulin sensitivity resulting from fat and protein intake. This implies a need for additional insulin in response to fat and protein intake; otherwise glycaemic control will be compromised. The aim of this study was to examine the effectiveness of calculating the insulin dosage required for an evening meal, taking into account the carbohydrate and the fat and protein content, in contrast to the usual concept of pure carbohydrate cover in adults with type 1 (T1) diabetes using insulin pumps. The study focused on pump-treated T1 diabetes patients.
Methods: Postprandial glucose values were examined over 12 hrs by continuous glucose monitoring (CGMS Medtronic), using a cross-over design. Fourteen insulin pump users took part in the study (10 females; age 39 ± 9 years (mean ± SD), diabetes duration 16.4 ± 6.7 years, insulin pump use 7.6 ± 3.3 years, HbA1c 7.3 ± 0.5%, BMI 26.4 ± 1.9 kg/m^2). Patients with comorbidities or diabetes related complications were excluded. Participants received the same test meal (meat, potatoes, salad and vanilla ice-cream) on three successive evenings. The insulin doses were calculated using 5.5 carbohydrate unit (1 carbohydrate unit = 10g carbohydrate) and 5.5 fat protein unit (1 fat protein unit (FPU) = 100kcal fat and protein). The insulin cover for the carbohydrate was a standard bolus (100% fast-acting) and a dual bolus (50% fast, 50% delayed over 8 hrs). As additional cover for fat protein unit the same insulin amount was used as for a carbohydrate unit. The insulin for the carbohydrate was administered immediately before the meal (quick acting) and for

the fat protein unit delayed over 8 hrs (FPU bolus). Compared were the area under the curve (AUC) as a numerical integral from 145 (1 preprandial, 144 postprandial) sensor glucose values, as well as the number of values under (<80 mg/dl), within (80-140 mg/dl) and above (>140 mg/dl) the target area from the sensor glucose values.
Results: The FPU bolus in comparison with the standard bolus led to a significant improvement in the AUC (22.399 ± 5.909 versus 25.419 ± 6.139, p=.02) and highly significant both within (67 ± 51 versus 34 ± 36, p<.01) and above (74 ± 52 vs. 108 ± 40, p=.01) the target area. For carbohydrate only based insulin dosage, no significant difference could be shown between the standard and the dual bolus; this applies to the total area under the curve and all 3 target areas from the sensor glucose values (25.419 ± 6.139 versus 25.292 ± 5.399, under 3 ± 7 versus 1 ± 2; within 34 ± 36 versus 31 ± 26, above target 108 ± 40 versus 108 ± 40; all p-values >.5). Regarding the comparison between FPU bolus and dual bolus, significantly more values lay within (67 ± 51 versus 31 ± 26, p=.04) and significantly fewer values above (74 ± 52 versus 108 ± 40, p=.03). The FPU bolus did not result in a difference for the lower target area (3 ± 6 versus 1 ± 2, p=.62).
Conclusion: The study therefore suggests that the therapy of T1 diabetes patients should consider insulin cover for the fat and protein portion of a meal as well as the carbohydrates, with the aim of optimising postprandial glucose values and glycaemic control. The study should be repeated in a larger group of insulin pump users with T1 diabetes.
Supported by: Abbott Diabetes Care

1011

Should the amount of fat and protein be taken into consideration to calculate preprandial insulin bolus?
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Background and aims: Continuous subcutaneous insulin infusion (CSII) provides the most physiological way of insulin administration as we can program different basal rates and deliver different types of insulin boluses. Traditionally theses boluses are calculated according to the amount of carbohydrate (CH) eaten. However, there are very few data published related to changes in glycemic response according to meal composition(only carbohydrates (CH) or CH with protein and fat). Our aim is to determine whether the presence of protein and fat could involve a different postprandial glycemic response than that obtained with only CH.
Materials and methods: Observational prospective study. 19 type 1 diabetic patients (for at least 2 years) on CSII (for at least 3 months) wore a blind continuous glucose monitoring system sensor (Minilink real time, Medtronic) for three days. They ingested on different days two meals with the same CH content but different fat and protein content. Mean A1c was 7.7% (range 6.5-8.4%) and mean age was 34 years old (range 30-52).Exclusion criteria: celiac disease or any gastrointestinal disease, any diabetes complication and any medication that could modify gastric emptying. Our protocol was next:
- First day: The sensor was inserted and calibrated
- Second day: Patients ate meal 1: 60g of pasta + 50ml of tomato sauce (meal composition: 50g CH, 3.3g of protein and 8.9g of fat)
- Third day: Patients ate meal 2: 60g of pasta + 50ml of tomato sauce + 150g of veal chop + 10ml of olive oil (meal composition: 50g of CH, 28.9g of proteins and 37.4g of fat)
- Fourth day: Sensor was removed

During the monitoring, alcohol intake and exercise were not allowed. They performed one self-monitoring capillary blood glucose per hour and remained in rest for a least 3 hours after each meal. They used the standard single-wave insulin bolus based on each subjects´s CH- to- insulin ratio and CH counting. Once downloaded the CGMS we analysed:
- Mean glucose 60 min pre-meal (Gm)
- Glucose Standard desviation (SD)
- Area under the curve described from the beginning of the meal until the glucose returned to levels pre-meal (AUC)
- Time (minutes) until glucose returned to values pre-meal (Tn)
- Maximum glucose peak (P)
- Time (minutes) to reach the maximum glucose peak (Tp)<br

A p value <0.05 was considered statistically significant.
Results: Patients started both meals with similar glucose levels. There were no statistical differences between both meals in Tn, AUC, P and Tp (Table 1). The greatest glucose peak was reached at 60 min and 79 min with meal 1 and 2 respectively.

Conclusion: The presence of protein and fat did not determine a different glycemic response. Although a delayed postprandial glucose peak was found in meal 2, this did not reach statistical significance. According to our results, there is no need in using a different insulin bolus when we ate an equilibrated amount of CH, fat and protein in a meal.

Table 1. Gm, AUC, Tn, P and Tp with meal 1 and 2

	Meal 1	Meal 2	p value
Mean Gm (mg/dl)	125.5	124	0.8
Mean AUC (mg/dl.min)	28.1	32.3	0.7
Mean Tn (minutes)	104.6	114	0.9
Mean P (mg/dl)	50	58.6	0.7
Mean Tp (minutes)	59.6	79	0.6

Supported by: sanofi-aventis

PS 95 Mapping and improving diabetes control and complications

1012

The PANORAMA pan-European survey: glycaemic control and treatment patterns in patients with type 2 diabetes

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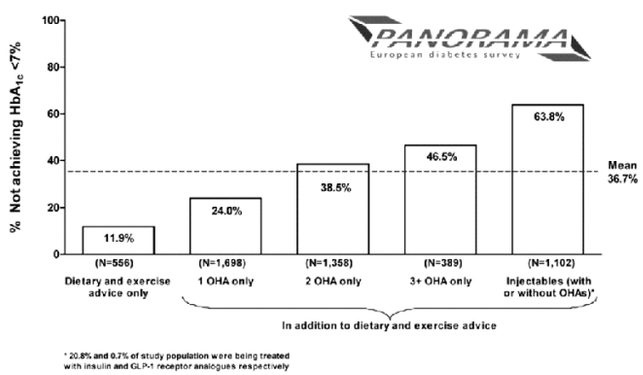
Background and aims: The prevalence of type 2 diabetes (T2D) continues to rise across Europe. Despite effective treatments initiated after diet/lifestyle modifications, many patients still do not achieve an HbA_{1c} target of <7%. PANORAMA is a large pan-European cross-sectional survey (NCT00916513) of patients with T2D treated with glucose-lowering therapies, aimed at assessing treatment satisfaction, quality of life and the proportion of patients achieving an HbA_{1c} <7%. This abstract reports recent data from 8 countries on glycaemic control in patients with T2D in relation to treatment patterns.
Materials and methods: Patients with T2D were randomly or consecutively selected from physician practices (mainly in primary care) in 8 countries (Belgium, Germany, Greece, Italy, Netherlands, Spain, Turkey and UK). Eligible patients: aged ≥40 y, with a diagnosis of T2D for >1 y prior to study entry and an available medical record at the clinic of >1 y. All patients received dietary and exercise advice. Most patients were also being treated with either oral hypoglycaemic agents (OHAs) or injectables (insulin and GLP-1 receptor analogues) with or without OHAs. Treatment type was unchanged in the previous 3 months. HbA_{1c} levels were measured using an identical portable diabetes monitoring system (Bayer's A1Cnow®) in each centre.
Results: 5,156 patients were included in the study from June to November 2009: 47.8% women; mean age 65.9 y (SD 10.3). Mean time since diagnosis: 9.0 y (SD 7.4). Patients were treated with advice only (10.9%), advice plus either 1 OHA only (33.3%), 2 OHAs only (26.6%), ≥3 OHAs only (7.6%), or injectables with or without OHAs (21.6%). Treatment patterns varied considerably between countries. Mean HbA_{1c} of the entire group was 6.9% (SD 1.1). However, 36.7% of patients did not achieve an HbA_{1c} <7%. The figure shows the percentage of patients in each treatment category who did not achieve the target HbA_{1c} <7%.
Conclusion: When comparing the PANORAMA survey results with previous data, it appears that the level of glycaemic control in Europe may be improving. However, there is still a gap between current management and optimal treatment of patients with T2D. Furthermore, the percentage of patients not achieving target HbA_{1c} increased as treatment was intensified. Possible explanations are that concern over hypoglycaemia may have delayed treatment intensification, reduced treatment adherence or patients on more intensive treatment may have had more difficult to control T2D perhaps due to a longer duration of T2D. These results suggest that earlier and/or more effective intensification of treatment may be needed to enable patients to achieve target HbA_{1c} as the disorder progresses.



Supported by: AZ & BMS

1013

The Mapping Glycaemic Control Across Australia (MGCAA) study: a population based national diabetes surveillance study

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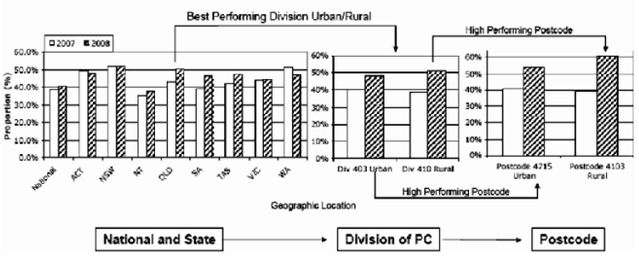
Background and aims: There are approximately 1.4 million Australians over the age of 25 with diabetes. More than half experience suboptimal glycaemic control, placing themselves at significant risk of complications and hospitalisation. Benchmarking glycaemic control is the first step in establishing a Diabetes Surveillance System, which allows for the development of targeted interventions to regions with the greatest need, as well as the subsequent monitoring of any intervention success. In Australia, previous studies have provided good quality data benchmarking glycaemic control at a national level. However, none have been able to benchmark, quantitate or qualify glycaemic control geographically at a national, state, Division of Primary Care and postcode level. Furthermore, none have been inclusive of demographic, clinical and biochemical markers. Considering the population diversity in a country as large and geographically diverse as Australia, such information is critical to the optimisation of diabetes resource management. The MGCAA study has achieved this.
Materials and methods: De-identified data including HbA_{1c}, lipids, age and gender were collected from private pathology laboratories. The data were cleaned (duplicates and screening HbA_{1c} values removed) and stratified geographically, providing a community population sample of approximately 250,000 diabetes patients. Demographic data including socioeconomic, lifestyle and cultural data were obtained and incorporated into the sample. The data were reported at a national, state and division of primary care regional level and made available via electronic mapping tools. Data from this first year of collection were regarded as benchmark data. Data were collected the following year and the cohort identified in year 1, were tracked and analysed, establishing a diabetes surveillance system. This is the first of a 5 year follow up period.
Results: Of the original 250,000 patient data collected in 2007, 56% had repeat data available in 2008 for follow-up (~140,000 patient samples), representing 10% of the estimated diabetes population of Australia. Although change in national glycaemic control was marginal (7.7% in 2007 vs 7.6% in 2008), there were clear regional differences in both mean HbA_{1c} as well the proportion of patients achieving HbA_{1c} targets (Fig 1). Regional differences were apparent with the proportion of these patients achieving HbA_{1c} glycaemic targets of <7% increasing in 4 states, remaining the same in 2 states and decreasing in 2 states.
Conclusion: The diabetes surveillance system can be interrogated to identify trends in glycaemic control, regional differences and those Divisions of Primary Care and the associated postcodes with the greatest glycaemic improvement (Fig 1) as well as those with the greatest need. Strategic interventions incorporating processes identified from areas with the greatest success can then be implemented and effectiveness monitored through prospective surveillance.

Fig 1: Proportion of Patients Achieving HbA_{1c}<7% per Geographic Region



Supported by: Novo Nordisk

1014

Development in quality of treatment and changes in treatment regimens in complicated type 2 diabetes

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Background and aims: Optimal treatment of Type 2 Diabetes (T2D) relies on a multifactorial approach. Approximately 1800 patients with T2D are con-

trolled at the outpatient clinic at Steno Diabetes Center (SDC), which is a specialized diabetes hospital in Denmark. A high proportion of these suffers from one or more complications, i.e. app. 60% have micro or macro-albuminuria, app. 9% have undergone retinal laser treatment and app. 30% suffers from severe neuropathy. The purpose of this study was to assess to which degree the treatment goals as defined by ADA and documented in the Steno 2 study published 2003 is achieved, before and after the publication of these results.

Materials and methods: Patients treated at SDC have since 2001 all been registered in an electronic patient record system. All patients with T2D who have attended the outpatient clinic for ≥6 month in the years 2002, 2006 and 2009 were identified. The proportion of patients reaching ADA treatment goals with regard to HbA1c, blood pressure (BP) and lipids was identified as shown in the table. Furthermore, the proportion of patients receiving different antidiabetic treatment regimens (oral antidiabetics (OAD) only, insulin only or a combination of OAD and insulin) was identified.

Results: The survey shows that it was possible to achieve the treatment goals in the majority of the patients with regard to lipid levels and diastolic BP, whereas this was not the case with regard to systolic BP and HbA1c. However, it was possible to approximately double the proportion of patients achieving the treatment goal of HbA1c in the years 2002 to 2006. In the same period the proportion of patients receiving a combination of OAD and insulin increased by 13%, whereas the proportion of patients receiving insulin monotherapy decreased by 10%. The proportion of patients treated with either diet (3-4%) or OAD only (19-20%) was unchanged throughout the period. With regard to BP lowering agents the proportion of patients receiving two or more agents increased from 55 to 67% in the period whereas the proportion of patients receiving lipid lowering medication increased from < 40% to >80% (data not shown).

Conclusion: The results suggest that in patients with complicated T2D it is possible to achieve near optimal results with regard to lipid levels and diastolic BP and acceptable control with regard to systolic BP. With regard to glucose control, it is possible to markedly increase the proportion of patients achieving treatment goal, however, even more focus is needed.

Changes in treatment regimens and achievement of clinical goals provided in percentages of patients

Year	Treatment with OAD only	Treatment with insulin only	Treatment with OAD +insulin	HbA1c ≤ 7.0%	Diastolic BP ≤ 80 mmHg	Systolic BP ≤ 130 mmHg	Total cholesterol ≤4.5 mmol/l	LDL cholesterol ≤2.5mmol/l
2002	20	48	28	15	60	27	35	44
2006	19	38	41	28	68	27	70	80
2009	20	39	39	25	66	38	71	79

1015

Socio-economic status, incidence of type 2 diabetes and relative mortality in Scotland 2001-2007

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Background and aims: Relative risks of mortality associated with type 2 diabetes (T2DM) have declined in recent years but are higher in women than men in many populations. The role of socio-economic status (SES) in risk of mortality among people with diabetes is not clear.

Materials and methods: We used data from a population-based national diabetes register to investigate the associations between T2DM, socio-economic status (SES) and mortality. SES was categorised with Q5 and Q1 representing the most deprived and most affluent quintiles from an area-based measure. Age-standardised incidence for 2004 and relative risks (RR) for all-cause mortality among people with incident T2DM of 35 to 84 years of age between 2001 and 2007 were estimated using general population data, the European standard population and Poisson regression models.

Results: Complete data were available for 111,441 people who developed type 2 diabetes between 2001 and 2007 and there were 8,775 deaths before the end of 2007. SES had a more marked effect on age-standardised incidence among of T2DM women (717.5 vs 357.2 per 100,000, age-adjusted RR for Q5 vs Q1 (95% confidence interval [CI]) 1.91 (1.62-2.25)) than men (comparable estimates 918.6 vs 568.9 per 100,000, 1.59 (1.38-1.84)). Age and SES adjusted RR (95% CI) for mortality were 0.97 (0.93 to 1.01) for men and 1.11 (1.07 to 1.16) for women. Age and sex adjusted RR for mortality associated with type 2 diabetes was lower for Q5 (0.93 (0.89-0.97)) than for Q1 (1.19 (1.12 to 1.27)).

Conclusion: Relative risks for mortality associated with incident T2DM were lower in this population than reported in previous studies. Incident diabetes was not associated with increased mortality among men but was associated with higher mortality in women compared to women without diabetes. SES modifies the effect of T2DM on mortality but does not explain sex differences in RR. Further work is required to establish whether these findings can be explained by risk factor patterns.

Supported by: Scottish Government, Scottish Health Informatics Programme

1016

The national inpatient diabetes audit reveals poor levels of inpatient foot care

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Background and aims: The standards of expected inpatient foot care have been recently outlined in the document "Putting feet first" however there has been no previous national assessments of the burden or quality of inpatient foot care.

Materials and methods: A national audit of clinical care was undertaken in acute hospitals in the UK on a single day between the 21st and 25th of September to determine diabetes prevalence, quality of care and patient experience. The audit form included key questions related to areas of foot care. The National Diabetes Inpatient Audit Day database was analysed to assess the standard of foot care.

Results: 14,259 patients in 219 hospitals were audited. 11.6% had a past history of foot disease. When diabetes was the primary reason for admission a foot problem was the most frequent diagnosis (24.9%), yet only 79% were referred to the foot team. 26% of hospitals did not have a multidisciplinary foot team. Mean length of stay was 22 days for those admitted with a foot complication compared with 15 days for other diabetes related admissions. Only 34% of patients recalled a visual foot inspection and only 30% a physical foot examination. In-hospital foot complications developed in 3%.

Conclusion: The figures are concerning. The audit confirms the significant inpatient burden of foot disease but also a disturbingly poor level of foot care. The results support the urgent need to implement foot awareness programmes such as "Putting Feet First".

Supported by: NHS Diabetes, Leicester, UK

1017

Clinical inertia in patients with type 2 diabetes and poor glycaemic control in a multicentric sample of patients cared for in primary in Catalonia (Spain)

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Background and aims: To assess clinical inertia (the providers' failure to increase therapy when treatment goals are unmet) in patients with type 2 diabetes (DM2) cared for in primary care.

Materials and methods: A multicentric cross-sectional study. Clinical inertia, defined as absence of modification of the treatment in patients with HbA1c> 7 %, was assessed in a random sample of patients with DM2 cared for in 52 primary care centres of Catalonia (Spain) in 2007.

Results: A total of 3130 patients were evaluated of whom 2783 had some HbA1c value. Of these, 997 had an HbA1c>7 %; 51.1 % males; mean age 67.1 years, standard deviation (SD) 12.1; 8.2 years of diabetes evolution-SD 6.3. Some changes were made in 66.8% patients: insulin dose increase in 40.5%, the addition of an oral agent in 45.8% or the start of insulinization in 3.7%. The mean value of HbA1c in patients for whom treatment was changed was 8.4%. Clinical inertia was observed in 33.2% of patients (CI95% 30.31-36.1); decreasing with the complexity of treatment: diet 38,8% (CI95% 20.2-57.4%), a single oral drug 40.3% (CI95% 35.2-45.4), two or more oral agents 34,5% (CI95% 23,3-45,7), insulin in monotherapy 26.1% (CI95% 18,2-34.0) and insulin plus oral agents 21.4% (CI95% 20,9-33,3). Clinical inertia decreased

as HbA1c increased: 37.3% (CI95% 33.2-41.4) between 7.1 and 8%; 29.4% (CI95% 23.5-34.9) between 8.1 and 9% and 27.1% (CI95% 20.9-33.3) if ≥9%. There were no differences in the characteristics of the patients in which the treatment was modified or not. The greatest opportunity for improvement lies in patients treated with diet or oral monotherapy (40.5%)
Conclusion: Clinical inertia affects a third of diabetic patients with poor glycemic control and is more related to a close to objective HbA1c value than to the complexity of the treatment or the patient characteristics. The changes are introduced with a mean HbA1c well above the therapeutic goal.

1018

Poor glycaemic control in secondary care insulin treated patients correlates with bad process indicators

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Background and aims: Evidence based medicine and quality control systems drive diabetes care, but room for improvement, not only in glycemic control, but also in follow up of other outcome and process indicators, exists. In the present study we examine how glycemic control is related to other outcome and process indicators.
Materials and methods: We used the 2009 data from a Belgian quality assurance study that has been carried out since 2001 in all hospital-based diabetes centres (n=113) and provides data (demographics, blood glucose control, cardiovascular risk status, diabetes complications, self-monitoring, and drug treatment) on a cross-sectional random 10% sample of the adult type 1 and type 2 diabetes patients on ≥ 2 daily insulin injections. Logistic regression analysis was used to examine the relationship of HbA1c with 5 process and 5 outcome indicators, while adjusting for age, diabetes duration and gender.
Results: In the type 1 diabetes population (n=3407; 57% males) the median age, diabetes duration and HbA1c were 47 years, 17 years and 7.8%, respectively. In the type 2 diabetes population (n=7879; 49% males) the median age, diabetes duration and HbA1c were 69 years, 14 years and 7.5%, respectively. Table 1 shows the performance in terms of process and intermediate outcome by HbA1c and diabetes type (Table legend: (1) $p<0.05$; (2) $p<0.01$; (3) $p<0.001$: Results from logistic regression analysis, after adjustment for age, gender and diabetes duration. HbA1c < 7% is used as reference.). Especially in type 2 and to a minor extent in type 1 diabetes, patients with the worst glycemic control (HbA1c ≥ 9%) were significantly less likely to be screened for complications (except for microalbuminuria screening) than the patients with optimal glycemic control (HbA1c < 7%). In both diabetes types, patients with suboptimal glycemic control (HbA1c ≥ 7%) were significantly less likely to reach blood pressure and blood lipid targets compared to patients with optimal glycemic control. Moreover in type 1 diabetes the proportion of smokers increased significantly with increasing HbA1c. These results were independent of age, diabetes duration and gender.
Conclusion: Quality of care in this population of diabetes patients with advanced disease stage was relatively good in terms of process and intermediate outcome. However suboptimal glycemic control was found to go hand in hand with poorer results for both other outcome and process indicators. The identification of patients characterised by this cluster of poor performance and of the causal factors merits further investigation.

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Fifteen years of improvement in process and outcome indicators in the management of type 2 diabetes mellitus in primary care centres in Catalonia, Spain

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Background and aims: To assess the evolution of the quality indicators of type 2 diabetes care in Primary Health Care centres in Catalonia over 15 years (1993-2007).
Materials and methods: A multicentric cross sectional study with the same sampling methodology in 7 evaluations. Continuous quality improvement program of GEDAPS (Primary care Group for the study of Diabetes) from the Catalonian Society of Family and Community Medicine. Assessment of the process and outcomes indicators in a randomized sample from each centre. Number of participating centres: 57 in 1993, 75 in 1995, 75 in 1998, 78 in 2000, 96 in 2002 and 52 in 2007. Sample sizes: 2239 in 1993, 3532 in 1995, 4217 in 1998, 45667 in 2000, 5819 in 2002 and 3130 in 2007.
Results: We observed a significant improvement in the following process indicators: foot examination from 49% (CI95% 46.9-51.1) to 64% (CI95% 62.3-65.7); laboratory measurements: HbA1c from 69% (CI95% 67.1-70.9) to 89% (CI95% 87.9-90.1), cholesterol from 76% (CI95% 74.2-77.8) to 91% (CI95% 90.0-92.0) and albuminuria from 34% (CI95% 32.0-35.9) to 59% (CI95% 57.3-60.7). A significant improvement in intermediate outcome indicators was seen in glycemic control (HbA1c <7%) from 39% (CI95% 37.0-41.0) to 65% (CI95% 63.3-66.7) and cholesterol (total cholesterol <200 mg / dl) from 26% (CI95% 24.2-27.8) to 61% (CI95% 59.3-62.7). There was no change in the strict control of blood pressure (BP <130/80 mmHg) 22% vs 21%, but an improvement was seen with a less stringent criteria (BP <140/90 mmHg) 45% (CI95% 42.3-47.1) vs 57% (CI95% 55.3-58.7). We observed a significant reduction in the prevalence of the following final outcome indicators: foot ulcer 7.9% (CI95% 6.8-9.0) vs 2.6% (CI95% 2.0-3.2), amputation 2.1% (CI95% 1.5-2.7) vs 0.6% (CI95% 0.3-0.9) and retinopathy 37% (CI95% 35.0-39.0) vs 15% (CI95% 13.7-16.2).
Conclusion: There were significant improvements in some of the process indicators, the glycemic, blood pressure and lipid control and a decrease in complications like retinopathy and diabetic foot.

	Type 1 diabetes				Type 2 diabetes			
HbA1c	**<7% (n=692)**	**7-7.9% (n=1119)**	**8-8.9% (n=891)**	**≥9% (n=677)**	**<7% (n=2473)**	**7-7.9% (n=2689)**	**8-8.9% (n=1589)**	**≥9% (n=1031)**
% Screening microalbuminuria	89	88	88	87	86	86	84	82
% Eye examination	85	87	87	80(2)	83	82	84	76(3)
% Foot sensation examination	88	87	85	84	81	81	81	77(3)
% Foot pulses examination	88	89	91	88	87	88	88	85(1)
% ≥ 3 HbA1c determinations/year	68	75(1)	70	60(2)	57	60	57	48(3)
% Blood pressure < 130/80 mmHg	40	32(2)	33(1)	32(1)	24	21(2)	19(3)	20(2)
% LDL < 100 mg/dl	63	59	52(2)	49(2)	65	66	62(1)	53(3)
% Cholesterol < 175 mg/dl	53	45(1)	42(2)	36(3)	60	58(1)	55(3)	46(3)
% BMI < 25 kg/m²	52	45	45	46	15	13	13	12
% non-smoking	84	82(1)	77(3)	67(3)	88	87	87	83

PS 96 Monitoring and delivering: practicalities for every day practice

1020

Use of an alcohol-based hand sanitiser did not affect readings of a blood glucose monitoring system

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Background and aims: People with diabetes mellitus (DM) are instructed to wash their skin with soap and water prior to self-monitoring of blood glucose (BG) to remove any dirt or food residue that might alter the reading. Alcohol-based hand sanitizers have become popular when soap and water are not available. The aim of this study was to determine whether the use of a common hand sanitizer has any effect on BG results from a meter system.
Materials and methods: We enrolled 34 non-fasting subjects (14 male/20 female, mean age 45(SD: 9.4)y, 2 with DM). Laboratory personnel prepared four separate fingers on one hand of each subject by: 1) washing with soap and water and towel drying (control finger); 2) cleaning with hand sanitizer (Purell® Instant Hand Sanitizer with Aloe, active ingredient 65% ethyl alcohol, Johnson & Johnson Consumer Products, Skillman, NJ) per instructions (Purell finger); 3) coating with cola (Coca-cola, Coca-Cola Company, Atlanta, GA) and air drying (cola finger); and 4) coating with cola and cleaning with Purell after cola dried (Purell after cola finger). Fingersticks were performed on each prepared finger and BG was measured with the OneTouch® Ultra® Blood Glucose Monitoring System (LifeScan Inc., Milpitas CA). YSI plasma glucose was also measured using the control finger. BG tests, shown in the table, were completed within 10 minutes.
Results: Mean BG values from the Purell finger and Purell after cola finger did not differ significantly from the Control finger (p=0.07 and 0.08, respectively). Cleaning with Purell resulted in 100% and 99% accurate readings versus YSI based on ISO 15197 and consensus error grid (zone A) analysis, respectively. Cola finger BG was substantially higher than the other skin conditions. In 16 cases, cola residue caused blood samples to smear and produce error messages and in 3 cases, cola residue caused BG values > 600 mg/dL (excluded).
Conclusion: In our study, cleaning with Purell hand sanitizer did not affect fingerstick BG tests performed with the OneTouch® Ultra® System.

Table: Glucose Mean (SD), N, and Finger Condition after Preparation (as described).

Finger Skin Condition	N	Glucose, Mean (SD), mg/dL
Control finger	34	100.6 (16.5)
Purell finger	34	103.6 (17.1)
Cola finger	15	182.9 (102.8)
Purell after Cola finger	34	103.8 (17.4)

1021

Improving the reliability of capillary blood glucose monitoring: using the first or second drop of blood

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Background and aims: Self monitoring of blood glucose (SMBG) is an important tool to achieve good glycemic control. Several aspects concerning SMBG need attention. Eg, there is no general agreement regarding the use of the first or second drop of blood for glucose monitoring. Various international studies and recommendations, advise to use the first drop of blood, after washing hands with water and soap. Still, in daily practice patients can not or do not always wash their hands. Our aim was to investigate the influence of having clean or soiled hands on glucose concentration in the first and second drop of blood.
Materials and methods: Eligibility criteria were patients with diabetes mellitus type 1 (T1DM) or type 2 (T2DM), using insulin, age above 18 years. A cross-sectional, 'within subjects' design was used. Wilcoxon Signed Rank Test was used to test for differences in glucose concentrations. Capillary glucose concentrations were measured in two consecutive drops of blood in the following three circumstances. Firstly, without washing hands, secondly, after washing hands with water and soap, and thirdly, after soiling the fingers with apple or banana. Results were compared to a control measurement in each finger used in the study. Results were also assessed, looking at the frequency of differences ≥ 10 % higher glucose concentrations vs control.
Results: Recruitment took place between September 2009 and January 2010. Our study population consisted of 123 patients, 63 (51%) men, 66 (54%) T1DM patients, and 57 (46%) T2DM patients, mean age was 54,4 years (SD 14,2) mean HbA1c was 58 mmol/mol (or 7,5% SD 1,3) and mean BMI was 29 kg/m² (SD 6,2). The table shows glucose concentrations for the three different circumstances. Not washing hands led to more than 10% higher glucose concentrations in the first and in the second drops of blood in 10% of the patients (p<0.001). Fingers soiled with fruit led to more than 10% higher glucose concentrations in the first and in the second drop of blood in 91% and 13% of the patients, respectively (p<0.001 and p=0.002 respectively). After washing the soiled fingers, glucose concentrations were more than 10% higher in 2% and 4% of the patients, respectively. In 2 - 3% of the patients the glucose concentrations in the first drop of blood of clean hands were 10% higher than in the second drop of blood (not significant).
Conclusion: The first drop of blood of unclean hands should not be used to allow a reliable glucose measurement. Although wiping the first drop of blood of unclean hands improves readings considerably, in 10 - 13% of the patients the glucose concentrations are still 10% higher than the control measurement. The first drop of blood can probably only be used when the fingers are clean.

glucose concentrations (mmol/L) and median (interquartile ranges) in different circumstances

	first drop of blood	second drop	control
not washing hands	8.9 (6.4 - 12.6)	8.9 (6.5 - 12.3)	8.7 (5.9 - 12.2)
washing hands	8.5 (6.3 - 12.2)	(is control)	8.7 (5.9 - 12.2)
soiled finger	15.0 (10.5 - 21.7)	8.8 (6.5 - 12.4)	8.9 (6.4 - 12.3)
washing hands after soiling the finger	8.3 (6.3 - 11.9)	8.2 (6.3 - 12.0)	8.6 (6.2 - 12.0)

Supported by: sanofi aventis, Roche Diagnostics Nederland BV

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The effect of haematocrit on the results of measurements using glucose meters based on different techniques

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Background and aims: Hematocrit (HT) affects the measurement accuracy of glucose meters. Glucose concentrations measured in samples with high HT values are decreased whereas in samples with low HT are increased as compared to the laboratory method. This effect caused mainly by plasma displacement may vary between different devices. The aim of the study was to evaluate the effect of HT on glucose meter assays based on different measurement techniques.
Materials and methods: We studied glucose meters utilizing the glucose dehydrogenase reaction and four measurement techniques. The HemoCue (HemoCue AB) uses colorimetry, the Accu-Chek Active (Roche Diagnostics) - reflectometry, The Optium Xido (Abbott Diabetes Care) - amperometry and the Optium Omega - coulometry. EDTA venous blood samples with glucose concentrations ranging from 40 to 412 mg/dL were used. We modified the samples HT by adding or removing defined aliquots of plasma. Glucose concentrations measurements were performed using each evaluated meter in 27 batches, each containing 5 blood samples with HT amounting to 20%, 30%, 40%. 50%, and 60%. Altogether, 540 glucose assays (108 batches) were performed.
Results: A significant relationship between HT and glucose reading in all meters was found - the relative decrease in glucose concentration per 1% increase in the HT value amounted from 0.51% for Optium Omega to 1.28% for Optium Xido (p<0.0001). Moreover, for all meters, except Optium Xido, there was a significant modification of this relationship by glucose level. The mixed effects model after logarithmic transformation of glucose concentra-

tions, stratified by glucose level (200 mg/dL) showed a significant (p<0.0001) relative decrease in glucose level per 1% increase in HT for all meters in all strata ranging from 0.30% for Optium Omega in stratum <100 mg/dl to 1.32% for HemoCue in the same stratum. According to the ADA recommendations, the glucose meter error should not exceed 5%. In <100 mg/dL stratum this threshold would be reached after about 3.6% to 4.1% HT change in all meters except of Optium Omega, for which a change of 16.6% would be needed (p<0.0001). In 100-200 mg/dL stratum Optium Xido reached the error threshold after 4.2% change in HT, compared to values in the range of 7.9% to 11.1% for other devices (p200 mg/dL stratum, similarly, Optium Xido reached this threshold after 3.9% change in HT (p<0.0001), while for the other meters it varied from 7.2% to 12.5%.

Conclusion: There is a significant continuous effect of HT on measurement accuracy of glucose meters across the wide range of HT values and glucose concentrations. Amperometric measurements seem to be more sensitive, while the coulometric technique appears to be less sensitive to the HT interference than photometric techniques.

1023

Use of GDH-PQQ glucose meter systems in patients receiving maltose-containing therapies

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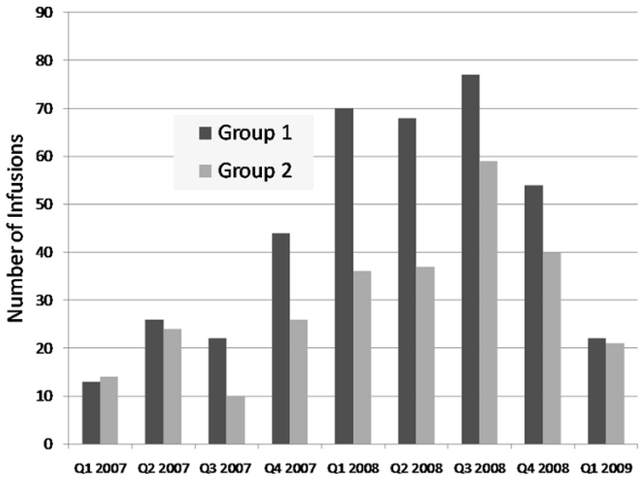
Background and aims: Blood glucose measurement (BGM) systems utilizing the enzyme, glucose dehydrogenase pyrroloquinoline quinone (GDH-PQQ), have lead to falsely elevated "glucose" readings in patients on medications containing maltose or a maltose precursor. Examples include icodextrin (a peritoneal dialysate metabolized to maltose), and OCTAGAM® and abatacept, which contain maltose as excipients. Over the past 10 years FDA's MAUDE Database and the literature have included multiple reports of severe hypoglycemia and death related to overtreatment with insulin or delayed detection of hypoglycemia, because of falsely high readings on GDH-PQQ meters. We reviewed a payor database to assess how frequently patients receiving OCTAGAM or abatacept also use a GDH PQQ meter system . Data were not available for peritoneal dialysis and limited for other maltose-containing therapies.

Materials and methods: The analysis used the Thomson Reuters Marketscan Database (years 2007 through Q1 2009), a large U.S. administrative claims database representing about 35.7 million employed, commercially-insured persons. We queried for persons with diabetes using glucose meters. This population was divided into three groups: Group 1 - Patients using only GDH-PQQ meters, Group 2 - Patients using only non-GDH-PQQ meters, and Group 3 - Patients using both GDH-PQQ and non-GDH-PQQ meters. The query identified patients who had received abatacept or OCTAGAM and ordered BGM test strips during the same quarter. As each quarter in the database is updated over time, the most recent quarters may be incomplete.

Results: The search identified 798,110 patients ordering BGM test strips during the two and one-quarter year period: 40.7% in Group 1, 54.7% in Group 2, and 4.6% in Group 3. Of these patients, 366 had also received abatacept or OCTAGAM infusions: 43.2% in Group 1, 45.6% in Group 2, and 11.2% in Group 3. Including those not using BGM, 5503 patients had received abatacept or OCTAGAM. The chart below compares the number of infusion events per group over the period Q1/2007 - Q1/2009. The majority (60%) of infusions were associated with Group 1 vs. 40% for Group 2. During the time period of observation, no decrease in the proportion of infusions associated with GDH-PQQ meters has occurred.

Conclusion: This study demonstrated that patients with diabetes on therapy with either abatacept or OCTAGAM frequently use GDH-PQQ meters, which are susceptible to maltose interference. Despite labeling (on both BGMs and medications) and FDA safety alerts, there is evidence that health care professionals and patients continue to prescribe and use, respectively, BGM systems susceptible to maltose interference despite patient exposure to therapies containing maltose. While use of abatacept and OCTAGAM is limited, the common usage of GDH-PQQ meters by patients taking these medications puts them at risk for obtaining falsely elevated blood glucose results that may contribute to or delay detection of hypoglycemia.



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Comparison of insulin diluent leakage post injection using two different needle lengths and injection volumes in obese patients with type 1 or type 2 diabetes mellitus

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Background and aims: Although the current market offers several different needle lengths for the injection of insulin, obese patients with diabetes are often advised to use longer needles (≥8-mm). Smaller, shorter needles have been shown to be safe and effective for insulin delivery while reducing patient discomfort. This study compared injections with 5-mm needles to injections with 8-mm needles regarding leakage, blinded pain, bleeding, and bruising at abdominal injection sites in obese patients with diabetes.

Materials and methods: Patients (n=56; 54% male; mean age=56; mean BMI=36 kg/m^2; insulin pen-naïve) with type 1 (n=13) or type 2 (n=43) diabetes participated in a randomised feasibility trial with patients blinded to needle length. Patients were injected by the investigator 3-5 times (to obtain 3 injections without bleeding to accurately measure leakage) in the abdomen with either 20 U equivalent (200 µL) volumes or 60 U equivalent (600 µL) volumes of sterile insulin diluent with both a 5-mm and 8-mm needle using a conventional insulin pen injector, HumaPen® Memoir™. The primary objective was to evaluate leakage at the injection site (by blotting with filter paper and weighing the filter paper using a tared, calibrated analytical balance) following injections (20 U and 60 U equivalent volumes) with 5-mm and 8-mm needles using a 4-step gatekeeping strategy. The success criterion for each gatekeeping step was defined such that the upper limit of the 90% CI for median leakage was <5% of the injected volume. Non-inferiority of 5-mm needle leakage compared to 8-mm leakage was evaluated with similar methodology. Secondary objectives were to compare injections (20 U and 60 U equivalent volumes) with 5-mm and 8-mm needles for blinded pain, bleeding, and bruising at injection sites.

Results: Leakage with the 5-mm needle for both the 20 U and 60 U equivalent volumes was <5% of the total volumes and was non-inferior to the 8-mm needle (see table). Pain scores were numerically similar for both needles (see table). Proportions of injections with bleeding (5-mm/20 U, 10.5%; 5-mm/60 U, 4.9%; 8-mm/20 U, 5.8%; 8-mm/60 U, 6.6%) and proportions of patients with bruising (5-mm/20 U, 8.1%; 8-mm/20 U, 10.8%, p=0.6; 5-mm/60 U, 21.1%; 8-mm/60 U, 26.3%, p=0.7) at injection sites were similar.

Conclusion: The results of this study support the suitability of the 5-mm needle for the injection of insulin in obese patients with diabetes with regard to leakage, pain, bleeding, and bruising at abdominal injection sites.

Needle Length (mm)		5	5 minus 8*	5	5 minus 8*
Injection Volume (U)		20	20	60	60
Leakage (U)‡	Gatekeeping† Step	1	2	3	4
	N	37	37	18	18
	Mean	0.0734	0.0094	0.1389	0.0820
	Median	0.0372	0.0075	0.0351	0.0185
	SD	0.0830	0.1064	0.1968	0.1605
	90% CI^	(0.024, 0.0569)	(-0.004, 0.017)	(0.0116, 0.126)	(-0.0113, 0.097)
Needle Length (mm)		5	8	5	8
Injection Volume (U)		20	20	60	60
Pain#	N	37	37	19	19
	Mean	1.27	1.14	1.68	0.95
	SD	2.76	1.70	2.45	1.58

* Difference in leakage between 5-mm and 8-mm needles
† The "gatekeeping" approach requires all previous tests to demonstrate a statistically significant result at the 0.05 level before analyzing the next test in the list
‡ 1 U = 10 μL = 10,000 μg
^ The CI was calculated for both the median and median difference (5 minus 8) using exact order statistics
# 0-20 point Visual Analog Scale (VAS)

Supported by: Eli Lilly and Company

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Safety evaluation of a new reusable insulin pen (ClikSTAR®) in 2526 Canadian patients (pts) with type 1 and type 2 diabetes mellitus (DM) receiving insulin glargine

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Background and aims: Insulin pens have increased the convenience, accuracy and flexibility of insulin therapy in comparison with traditional vial and syringe administration. ClikSTAR® is a novel reusable insulin pen developed for use with insulin glargine and insulin glulisine. The aim of the study was to assess the safety profile of ClikSTAR® after 6-12 weeks of use in type 1 & type 2 DM pts receiving insulin glargine.
Materials and methods: In this open-label, multicenter, non-randomized, uncontrolled, observational, prospective study pts with type 1 & type 2 diabetes receiving insulin glargine were eligible to participate. At baseline all pts received the ClikSTAR® pen, instruction leaflet and a free-toll number for Technical Support and had been trained on the pen use with insulin glargine cartridges. Baseline information was collected at the first visit. A follow-up visit assessment was performed by investigators at 6-12 weeks to collect any Product Technical Complaint (PTC). PTCs could be reported throughout the study and investigators used a decision tree guide to handle PTCs. All pens associated with a PTC were tested for Product Technical Failure (PTF) at the pen laboratory in Frankfurt. The risk acceptance criterion was that no validated PTF leading to a Serious Adverse Event (SAE) would occur within 2500 pts during a period of 6 to12 weeks.
Results: 2526 pts were enrolled (56% male and 44% female) in 104 sites across Canada, average age was 56 (SD = 14) years, 31% were type 1 DM and 69 % type 2 DM. 93% of pts were pen users prior to study enrolment. 9% and 8% had visual and dexterity impairment respectively. A total of 84 PTCs, 24 SAEs and 85 non serious adverse events (AEs) were reported. 3 pts with SAEs and 9 with AEs were reported as having a causal relation with a PTC. Investigation results of PTCs associated with an SAEs showed that they were not due to a pen failure. A total of 75 (3%) pts withdrew from the study; 15 for a PTC and 19 for an AE. The most common PTCs were related to the plunger, the dose selector and insulin delivery. No PTF related to an AE or SAE identified throughout the study and most PTCs were due to handling error of the pen and not requesting additional information or assistance from their site personnel or the Call Center.
Conclusion: This first real life observational safety study had been designed to collect complaints and adverse events reported by pts with diabetes using ClikSTAR® pen and insulin glargine. PTCs were infrequent and No PTF related to an AE or SAE reported. Results show that this new reusable pen is safe to be used with insulin glargine.
Supported by: sanofi-aventis

PS 97 Education: in the right hands - an effective therapy for diabetes

1026

Rapid reductions in insulin requirements in patients with type 1 diabetes participating in the DAFNE (Dose Adjustment for Normal Eating) structured education programme

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Background and aims: The Dose Adjustment for Normal Eating (DAFNE) programme consists of 4.5 days of structured education for patients with type 1 diabetes (T1D), to enable them to adjust their own insulin doses as required. This programme has previously been shown to lower HbA1c, reduce the incidence of hypoglycaemia and improve quality of life. There is anecdotal evidence that these improvements are associated with significant reductions in insulin doses, suggesting that some are over-insulinised prior to the programme. However, to date no formal analysis of changes in insulin doses associated with participation in DAFNE training has been conducted. We sought to determine whether DAFNE training is associated with changes in insulin requirements.
Materials and methods: We conducted a retrospective cohort analysis of adults with T1D who attended DAFNE training in our centre between October 06 and December 09. The type and dose of insulin used at the pre-course assessment and on the fourth day of the DAFNE course were recorded. HbA1c at the pre-course assessment and the first measured HbA1c between 3 and 12 months after completion of the course were also recorded, as was the presence of clinically apparent lipohypertrophy(LH). Data are presented as means ± SD. A two-tailed t-test was used to compare pre- and post- DAFNE training variables.
Results: Over the three year study period, 335 patients underwent DAFNE training at our centre. Complete pre-course and follow-up data were available for 259 patients (77%) and these were included in the current analyses. 124(48%) were female, 97% were Caucasian, mean age was 41 ± 14 years (range 18- 74) and mean duration of diabetes was 19 ± 14 years. 43.1 % of patients had documented LH. There were significant reductions in daily total insulin (52 ± 22 to 44 ±16 units), quick acting insulin (28 ±14 to 24 ±9) and basal insulin (24 ±12 to 20 ± 10) on day 4 of DAFNE training compared to the pre-course doses (all $p<0.001$). The dose reduction was greater in patients with LH than in those with no LH (10.1 and 6.5 units, respectively). However, the insulin dose reductions were statistically significant even in those with no LH ($p < 0.001$). Most patients were using insulin analogues (Insulin Aspart 66%, Insulin Lispro 25%, Insulin Glargine 65%, Insulin Detemir 22%). We estimate that the 16% reduction in total daily insulin dose would lead to an annual reduction in insulin cost of approximately 90GBP (100 euro), based on current drug costs. Importantly, insulin dose reduction did not worsen glycaemic control, with a small trend for HBA1c to improve when first checked after DAFNE (8.4 ± 1.3 vs 8.30 ± 1.2 pre vs post-course, $p =0.11$).
Conclusion: We observed significant reductions in the short term in insulin requirements in T1D patients undergoing DAFNE training. Although it is possible that changes in physical activity during the course may have contributed, the changes seen likely reflect more appropriate insulinisation of these patients, which would account for reductions in hypoglycaemia. Our data suggest that patients with T1D who do not have access to structured education may be systematically over treated with insulin.

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Lifestyle intervention by group-based rehabilitation versus individual counselling in type 2 diabetes: 1-year follow-up

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Background and aims: To compare the longer term effectiveness of delivering lifestyle intervention to type 2 diabetes patients as either group-based rehabilitation in primary care or individual counselling in an outpatient setting.
Materials and methods: We randomised 143 patients with type 2 diabetes to a 6-months group-based rehabilitation programme, including patient education, supervised exercise, and diet intervention, or to a 6-months individual counselling programme. Follow-up time was 12 months after baseline. Outcome measures were glycated haemoglobin (HbA_{1c}), cardiovascular risk factors, quality-of-life (SF-36, Medical Outcomes Study Short Form 36-item Health Survey) and self-rated health (DSC-R, Diabetes Symptom Checklist - Revised).
Results: In the rehabilitation group there was a decrease in HbA_{1c} (-0.2%-point, 95%CI [-0.4 to 0.03]), systolic blood pressure (-6 mmHg [-9.3 to -2.5]), diastolic blood pressure (-4 mmHg [-6.3 to -2.4], weight (-2.2 kg [-3.2 to -1.3]), and waist circumference (-2.0 cm [-2.9 to -1.1]). In the individual group there was a decrease in HbA_{1c} (-0.4%-point [-0.6 to -0.1]), systolic blood pressure (-3 mmHg [-6.3 to 0.7]), diastolic blood pressure (-3 mmHg [-4.7 to -0.7]), weight (-1.6 kg [-2.6 to -0.7]), and waist circumference (-1.6 cm [-2.5 to -0.6]). Self-rated vitality, fatigue distress, physical functioning and cardiovascular distress improved over time ($P<0.05$) in the two groups combined. Repeated measurement analysis did not result in significant differences between the groups of *any* outcome.
Conclusion: There were no significant differences between the two groups over time, but improvements in HbA_{1c}, blood pressure, weight, waist circumference, self-rated vitality and fatigue distress in the two groups combined were significant. However, the resource use of the rehabilitation programme was twice as much as the individual programme.
Supported by: Jascha Foundation, National Board of Health, Danish Ministry of Health

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Randomised controlled trial of the DESMOND structured education programme for people newly diagnosed with type 2 diabetes: biomedical outcomes, psychosocial measures and illness beliefs at three years

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Background and aims: DESMOND is a structured group self-management education programme (6 hours contact time) for patients newly diagnosed with type 2 diabetes. A multi-site cluster randomised controlled trial showed that DESMOND is associated with benefits in illness beliefs, weight loss, physical activity, smoking and depression. A three year follow-up of the participants has been carried out to see if these benefits are sustained beyond 12 months and the results are reported.
Materials and methods: Participants in the original trial who were eligible for follow-up at 3 years were sent a postal questionnaire (illness beliefs, symptoms of depression, quality of life, physical activity and smoking) and asked to visit their practice for biomedical measures (HbA1c, blood pressure, weight, blood lipids, waist circumference).
Results: Of the 743 people eligible for follow-up, biomedical data were collected on 604 (81.3%) and questionnaire data on 513 (69.0%). No statistically significant differences in biomedical measures were seen at 3 years, although a trend to greater improvement across all biomedical factors, smoking and physical activity was seen in those who received the intervention, for example mean change in HbA1c -1.32 (-1.57,-1.06) versus -0.81 (-1.02,-0.59). The significant benefits in the intervention group across 4 out of 5 health beliefs seen at 12 months were sustained at 3 years. For example, those in the intervention group report higher illness coherence compared to the control group (0.93, 95% confidence interval (CI) 0.20 to 1.65, p=0.01). At 3 years there is a trend to lower depression scores in the intervention group (-0.29, 95% CI -0.74 to 0.15, p=0.19). There are no differences between the groups for the problems areas in diabetes scale or the measures of quality of life at 3 years.
Conclusion: A one-off structured group self-management education programme for patients with newly diagnosed type 2 diabetes showed significant improvements in weight and smoking status at 12 months with sustained positive changes in a range of illness beliefs at 3 years.
Supported by: Diabetes UK

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Fear of hypoglycaemia: low incidence in educated patients with diabetes mellitus

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Background and aims: Hypoglycaemia is the most frequent adverse effect of drug treatment of diabetes and can in worst cases cause death, either by itself or, for instance, when driving or operating heavy machinery. Hence fear of hypoglycaemia is a reasonable reaction but in which dimensions concerning ratio and intensity and what are the definite reasons? Novel contraction of diabetes and inability to handle could be the most likely assumption. Suffering from hypoglycaemia frequently in the past could be another reason. To find out exactly we studied the prevalence and intensity of fear in patients with diabetes mellitus type 1 and 2 in a German cohort.
Materials and methods: We assessed fear of hypoglycaemia (FOH) in 719 patients taking any diabetes medication (age 62.2years; time since diagnosis 16.3years; BMI 31.2kg/m²; HbA1c 7.31%) and treated at a university outpatient department using a standardised questionnaire. All patients had at least one structured education in the preceding 20 years. We used a scale from 1-6 to rate FOH (1: no fear at all; 6: extensive fear). The patients were grouped to no fear (score 1, 2) and fear (score 4-6). Score 3 was discounted to exclude indecisive or uncertain decisions. Clinical and laboratory data are drawn from the electronic patient record EMIL (http://www.itc-ms.de). HbA1c was DCCT adjusted.
Results: 118 patients (8.1%) stated to have FOH. 492 patients (68.4%) are unafraid of hypoglycaemia. Patients with FOH were more female (49.2 vs 35.8%; p=0.007), younger (58.6 vs 63.7y; p<0.001), had a higher HbA1c (7.47 vs 7.24%; p=0.026), higher overall lifetime incidence of severe hypoglycaemia (0.69 vs 0.26; p=0.005), more non-severe hypoglycaemia (0.92 vs 0.46 per week; p<0.001), a higher plasma glucose threshold for symptoms of hypoglycaemia (3.8 vs 3.5mmol/l; p=0.01), did more blood glucose self monitoring (26 vs 21 per week; p<0.001), had more insulin injections (3 vs 2 per day; p=0.006) and had predominantly diabetes type 1 (33.1 vs 19.9%; p=0.002). Furthermore patients with FOH had a lower WHO (Five) Well-Being Index (12 vs 16; p<0.001; max. score 25), an increased impairment by hypoglycaemia (4 vs 3; p<0.001; max. score 6) and more difficulties at work caused by hypoglycaemia (3 vs 1; p<0.001; max. score 6) compared to the ones with no FOH. No significant differences were observed regarding time since diagnosis (17.2 vs 16.3years), the kind of therapy differentiated into therapy with insulin and oral hypoglycaemic agents (84.4 vs 76.4%), the daily insulin dose (52.7 vs 47.1U/d), last BMI (30.9 vs 31.5kg/m²), incidence of severe hypoglycaemia during the last 12 months (0.08 vs 0.04), the social status (10 vs 11; max. score 21), when nobody was around while hypoglycaemia (46.2 vs 40.2%) or when it arose at night (46.7 vs 39.7%).
Conclusion: There are proportionally fewer patients with diabetes who suffer from noteworthy fear of hypoglycaemia. General reasons for this fear are more likely its consequences such as impairment or difficulties at work, than the conditions per se. It may also be related to a certain type of personality, based on or interrelated with a poor Well-being Index. Because the patients in this survey were a selection from a university outpatient department, it is unclear to what extent these results can be transferred to the general population in which fear could either be less frequent or even more, when, for example, less participation in educational programmes is considered.

1030

Coaching by a dietician: a cost-effective alternative to diabetes management?

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Background and aims: World prevalence of diabetes and associated burden of care and complications are rising. Cost-effective management alternatives are badly needed. Cardiovascular risk (CVR) management by dieticians is one alternative worth assessing over a long term. This 3-year randomized trial aimed to show that dietician-led therapy management with an endocrinologist, for purposes of annual follow-up and advice as needed, enables recommended diabetes outcomes and is less costly than regular care.
Materials and methods: Diabetic subjects (n=101, HbA_{1c}>7%) were randomized to a Dietician-Coached Group (DCG) or a Conventional Group (CG) with follow-up as usual by endocrinologists ± general practitioners. DCG met with coach every 3 months (physical and biochemical measures, exercise, diet, smoking cessation, hypoglycaemia recording, capillary glucose monitoring and motivation), had monthly follow-up phone calls with coach and a yearly endocrinologist follow-up. Variables were measured every 3 months for DCG vs. yearly for CG.
Results: At baseline, groups (DCG n=51/CG n=50) were similar in age (60±10/60±11 yrs), duration of diabetes (16±9/16±10 yrs), systolic and diastolic BP (sBP: 131±15/131±24; dBP: 74±9/77±10 mmHg), fasting plasma glucose (FPG: 8.8±3.2/8.4±3.3 mM), HbA_{1c} (8.1±0.9/8.1±1.1%), triglycerides (1.88±1.28/1.78±1.23 mM), LDL (1.94±0.70/2.03±0.64 mM) and total cholesterol/HDL (3.39±1.16/3.50±1.27 mM). DCG subjects were heavier (BMI: 34±8/31±5 kg/m^2; p=0.03, waist circumference (WC): 113±18/106±12 cm; p=0.03). At 2 years (DCG n=39/CG n=42), ANOVA with repeated measures showed evolution between groups differed for HbA_{1c} (DCG: baseline: 8.1±0.9; 1 yr.: 7.3±0.8; 2 yrs.: 7.4±0.9% vs. CG: 8.1±1.1; 8.2±1.5; 7.8±1.1%; p≤0.001), dBP (74±9; 69±10; 69±10 vs. 77±10; 79±10; 76±9 mmHg; p=0.004), and WC (111±18; 110±18; 110±20 vs. 106±13; 108±13; 108±14 cm; p=0.015). At 2 years, DCG had an HbA_{1c} value of 0.4% lower than CG, which is clinically significant. Moreover, in DCG, the greater improvement in HbA_{1c} was observed at 1yr. vs. baseline (p≤0.001) compared to 2yrs. vs. 1yr. (p=0.08). There was no difference for BMI, FPG, microalbuminuria and lipid profile which was already on target at baseline.
Conclusion: After 2 years, diabetic patients coached by a dietician clinically improve HbA_{1c} and dBP. This simpler model of dietician coaching seems to be superior to regular care at improving diabetes outcomes and CVR factors. Should our 3-year analysis confirm our preliminary results? Cost analyses are ongoing.
Supported by: Pfizer

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Including community health workers in an effective model of diabetes self-management education

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Background and aims: 24 million people in the USA have diabetes, a costly chronic disease. Self-management education or training (DSME) is a key step in improving health outcomes and quality of life for these people. A team approach to providing DSME is described by the *Guidelines for the Practice of Diabetes Education* and is being adopted in practice. The aims of our study were to: a) define a model for DSME provided by a multi-level diabetes education team; and b) answer the question, "what are the unique roles and responsibilities of those who deliver diabetes education and care?"
Materials and methods: From 10/09-2/10, we conducted desk audits of 100 applications to the Diabetes Education Accreditation Program and examined 10 additional programs identified as having "best practices" to define the role of community health workers (CHW) in actual DSME practice and identify the education delivery models in which they worked. To be inclusive, the definition of CHWs included community health advocates, lay health advisors, lay health educators, community health representatives, tribal diabetes educators, peer health promoters, community health outreach workers, and promotores de salud. Findings from our review were compared to the National Standards for Diabetes Education and requirements put forth by the U.S. Centers for Medicare and Medicaid Services. These criteria were used to examine the strengths and weaknesses of the different approaches, roles and models identified.
Results: The review found that CHWs are frontline public health workers who are trusted members of and/or have a uniquely close understanding of the community served. CHWs can effectively serve as bridges between ethnic, cultural and geographic communities and the health care providers. There are four scenarios in which CHWs and experienced and/or credentialed diabetes educators can effectively work together. These four distinct DSME models are: 1) Shared Teaching; 2) Top Down; 3) Multi-Class at One Time; and 4) Supportive Role. In these models, the professional educator provides the clinical instruction and serves as supervisor. CHWs, who are non-professional health care providers with little expertise in DSME and/or management, support the DSME services provided to people with diabetes.
Conclusion: A DSME team approach is effective in helping people with diabetes gain the skill and knowledge necessary to change their behaviors and effectively manage their illness. Diabetes education is provided by health care professionals from many disciplines, involving practitioners with varying levels of experience/expertise in diabetes management, diabetes education, and clinical care. Moreover, DSME is most effective and sustainable when it is delivered by individuals who are prepared, competent, and function within the practice level articulated in the *Guidelines for the Practice of Diabetes Education*. Within the context of four models of DSME, and when supervised by a professional and/or credentialed diabetes educator, CHWs can effectively assist, provide important linkages to the local community, and successfully serve as part of the DSME team.

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An interactive 1 hour educational programme for junior doctors delivered at their induction improves the quality of inpatient diabetes care

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Background and aims: The prevalence of diabetes amongst the inpatient population is increasing and more rapidly than that in the general population as this represents a more aged population. This has increased the management decisions having to be made by junior doctors. Unfortunately postgraduate education does not routinely address the skills needed to appropriately manage this patient group and in hospitals in the UK relatively little time is given to teaching junior doctors about diabetes care.
Materials and methods: We designed interactive teaching programmes for medical and surgical junior doctors which can be delivered in 1 hour at their induction or within scheduled teaching. These were centered on adults learning theories. Key aspects of care were covered in a case based format. Reaction to the teaching sessions was evaluated using questionnaires. Learning was evaluated by assessing the change in the rating by trainees of their confident in managing 5 key areas of care assessed before and immediately after sessions. Whether this influenced practice was assessed by auditing aspects of inpatient care in the hospitals before and 3-5 months after completing education.
Results: 174 (85%) of 206 trainees provided feedback using Likert scales of 1-5. Clarity and ease of understanding scored highly (4.5) as did the response to the question of whether the designs of the programmes increase participation (4.5). The programmes were highly recommended (4.5). The most liked aspects were interactivity (21%), the case based format (22%) and teaching design (47%). Confidence in juniors' management in five key areas of care increased significantly [from 17.4 (SD 3.9) to 24.7 (SD 2.6), p < 0.001]. Following the teaching sessions the most common key take home points and planned changes in their clinical practice were related to improved glycaemic management (24 and 25% respectively), improved insulin infusion use (22 and 26%), avoiding prescription errors (20 and 22%) and foot assessment (19 and 13%). There were statistically significant improvements in the frequency of foot assessments, appropriate insulin infusion management and prescription errors (p < 0.05).
Conclusion: We have demonstrated that a well designed teaching programme on inpatient diabetes care can be effectively delivered within 1 hour and hence easily introduced to most hospitals. The interactive design, based on learning theories and using a case based format were likely to be central to the significant changes seen in trainees confidence; more importantly in the quality of inpatient care.

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Improving diabetes care through multidisciplinary training: The Christian Medical College-Vellore/WDF diabetes education project

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Background and aims: Diabetes is a major health care issue in India, yet doctors and nurses often have outdated information on standards of care, and foot care is underemphasized despite low rates of footwear use. We sought to improve diabetes care in rural and semi-urban areas by training doctors, nurses, foot care technicians (FCTs), and cobblers through an existing network of mission hospitals.
Materials and methods: A multidisciplinary training program was developed by the Departments of Endocrinology and Biochemistry at Christian Medical College-Vellore (CMC), the Schieffelin Institute of Health Research & Leprosy Centre (SIHRLC) and the Christian Medical Association of India (CMAI). Eligible hospitals were identified by the Dept of Endocrinology and CMAI. Each hospital was invited to send at least 1 doctor, 2 nurses, 1 FCT, and 1 cobbler to receive comprehensive training in diabetes care. Doctors were trained in a 10-day course and nurses in a 14-day course taught by professors and diabetes nurse educators from CMC's Department of Endocrinology, with support from other departments including Biochemistry, Rehabilitation and Ophthalmology. At SIHLRC, FCTs received 2 weeks of training in foot care and treatment of neuropathic foot problems, and cobblers received 1 month of training in orthopedic shoemaking. Key components of the program included emphasis on a team approach to diabetes care and the role of diabetes educators. Baseline data was collected from application forms. Evaluations were gathered for all teaching sessions. Follow-up questionnaires were sent by e-mail to all hospitals 6 months after completion of training. On-site monitoring visits were conducted for 20% of the trained hospitals.
Results: During 2004-2009, multidisciplinary training in diabetes care was provided for 100 mission hospitals in 22 states across India. At least 1 doctor was trained at 100% of hospitals, at least 1 nurse at 100%, at least 1 FCT at 58%, and at least 1 cobbler at 47%. Follow-up questionnaires were received from 36% of hospitals. Among those returning questionnaires, 89% had started diabetes clinics. There was an average of 53 new diabetes outpatients per hospital, an increase of 34% in patient capacity, by 6 months after training. Most hospitals (74%) joined CMC's laboratory quality control program. Other activities by trained hospitals included outreach clinics (25%), diabetes camps (39%), and health education (53%). These successes occurred despite significant attrition at some hospitals: trained doctors left at 17%; trained nurses at 35%; trained FCTs at 47%; and trained cobblers at 36%. Trained sites reported lack of staff as the most significant challenge they faced in providing quality diabetes care (74%), followed by the cost of care (53%) and lack of facilities (31%); difficulty procuring drugs was a relatively unimportant factor (6%).
Conclusion: Comprehensive, multidisciplinary training of doctors and nurses promotes the establishment of diabetes clinics in underserved areas and markedly increases the number of diabetic patients receiving treatment. Trainings should take attrition rates into account, and should emphasize low-cost treatment options.
Supported by: WDF

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Physician-patient ethnic concordance improves diabetes care for ethnic minority patients

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Background and aims: Non-White patients who receive care from physicians from the same ethnic group have greater satisfaction with care and improved health care utilisation. However, no studies have examined whether physician-patient ethnic concordance influences the quality of diabetes care, where greater cultural understanding may lead to improved dietary, physical activity and medication adherence. This study evaluated whether the quality of diabetes care for minority patients is influenced by the ethnicity of their family physicians.
Methods: Family physicians were randomly recruited from mutually exclusive neighbourhoods in the Toronto area with high concentrations of people with Chinese origins or people with South Asian origins. Recruitment was stratified by physician ethnicity. From each physician's practice, up to 10 diabetic patients with Chinese or South Asian origins were randomly selected. Diabetes quality indicators were collected by chart abstraction. The primary outcome was the last A1c achieved. Other outcomes were the last blood pressure and LDL-cholesterol achieved, and documentation in the chart of a foot examination. Within each ethnic group, outcomes were compared based on whether the physician was ethnically concordant or discordant with the patient. Generalised linear models were used to determine statistical significance, accounting for the clustered nature of the data within physician practices and adjusting for patient age, sex and diabetes duration.
Results: 45 family physicians were recruited and 416 patients were included. Most Chinese diabetic patients had Chinese family physicians. Chinese patients with Chinese family physicians achieved better glycaemic control than those with ethnically discordant family physicians. No difference in glycaemic control was seen among South Asian patients with ethnically concordant versus discordant family physicians, and no differences were seen in either group for other outcomes. There was a strong trend towards differences in foot examination rates, but because this outcome was highly clustered within physician practices, it was not statistically significant in either ethnic group.

Table: Quality of care for ethnic minority patients, stratified by physician ethnicity.

	Chinese diabetic patients			South Asian diabetic patients		
	Chinese physicians	Non-Chinese physicians	Adjusted p-value	South Asian physicians	Non-South Asian physicians	Adjusted p-value
n	148	22		129	117	
A1c	0.070±0.010	0.077±0.013	0.009	0.075±0.012	0.074±0.013	0.4
Systolic BP	129±12	128±14	0.6	130±16	127±16	0.3
Diastolic BP	76±8	78±8	0.2	76±10	79±8	0.2
LDL-cholesterol (mmol/L)	2.2±0.8	2.6±0.9	0.4	2.4±0.9	2.2±0.8	0.3
Foot examination	84%	59%	0.2	46%	57%	0.3

Conclusion: Among diabetes patients with Chinese origins, those whose family physician also had Chinese origins achieved better glycaemic control. These differences persisted even after adjusting for patient age, sex and diabetes duration. The absence of an effect in the South Asian population may be due in part to their greater linguistic and cultural heterogeneity compared to the Chinese population, or because there is greater English-language use among Canadians with South Asian origins than those with Chinese origins. The findings suggest that health care providers with better cultural understanding can influence diabetes care for patients from certain ethnic groups.
Supported by: Heart and Stroke Foundation of Ontario

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Effects of a systematic smoking cessation intervention in diabetic patients

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Background and aims: Cigarette smoking is the leading preventable cause of illness and premature death in developed countries. Several clinical studies have reported significant links between smoking and development of diabetes, micro- and macrovascular complications, and impairment of metabolic control. Although several studies have demonstrated the efficacy and cost-effectiveness of smoking cessation counseling in changing smoking behavior among the general population, the role of system-based approaches that make smoking a routine part of office contacts and provide multiple prompts, advice, assistance, and follow-up support in diabetic subjects remains to be clarified. Therefore, the aim of this study was to evaluate the effectiveness of a systematic smoking cessation intervention in diabetic patients as a routine component of diabetes care.
Materials and methods: All the afferent type 1 or type 2 diabetic patients at the Diabetes Center were systematically reviewed to confirm their smoking status. The diabetic subjects who were current smokers were considered

to be candidates for intervention. The intervention protocol consisted of: 1) assessment of dependence and motivation to stop smoking; 2) measurement of carbon monoxide concentration of expired air by smokerlyzer; 3) face-to-face interview with a nurse who was a member of the team; 4) optional nicotine replacement therapy or other smoking cessation medication; 5) follow-up support program which included a telephone call 2 and 4 weeks after the cessation date, a follow-up visit after 3-4 months and a final visit after 12 months.
Results: A total of 95 diabetic smokers were invited to participate to the intervention program and 82 (78%) agreed to take part in the study. After 6 months, 22 patients (27%) reported that they had stopped smoking and this was confirmed by measuring the carbon monoxide concentration of expired air. Among participants who continued smoking, a significant reduction was evident in the average cigarette consumption. Of the 82 patients participating to the intervention program 23% were treated with nicotine replacement therapy or varenicline and 28% were referred to smokers clinic for a more comprehensive evaluation.
Conclusion: The present study shows that a structured intervention that makes smoking a routine part of office contacts seems to be effective in inducing smoking cessation in diabetic patients, thus suggesting that a systematic intervention including smoking cessation counseling and other forms of treatment should be a routine component of diabetes care.

PS 98 Tools for improving diabetes control

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Design and development of a computer assisted clinical decision support system to help physicians manage patients with type 2 diabetes mellitus
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Background and aims: The increasing number of options for therapy and the failure of most patients to achieve A1C goals in a timely manner suggest the need for clinical decision support at the point of care. We sought to develop a customizable software system that could provide advice to primary care physicians at the point of care, providing recommendations and explanations.
Materials and methods: We have developed a computer-assisted decision support (CADS) system with customizable modules for patient, provider, and administrator. CADS is based on extensive statistical analysis and graphical displays of uploaded glucose data (SMBG); medication history; individualized glycemic goals; analysis of laboratory data (A1C, renal and hepatic function); and co-morbidities (cardiac, renal, hepatic, gastrointestinal). The software provides a concise report to the clinician regarding overall quality of glycemic control and identifies problems such as hypo- and hyperglycemia, excessive variability, insufficient glucose monitoring, and presence of various patterns. A rule-based expert system then makes recommendations to adjust dosage of current medications, discontinue medications, add new medications, or change the treatment regimen. We have constructed algorithms with recommended sequences for 61 regimens, including therapeutic lifestyle changes, 8 forms of monotherapy, 22 forms of dual therapy, and 30 forms of triple therapy utilizing 8 classes of medications: metformin, DPP-4 inhibitors, GLP-1 analogs, thiazolidenediones, sulfonylureas and glinides, alpha-glucosidase inhibitors, and basal insulins. Treatment pathways can be customized by the individual physician or clinic. The algorithm considers pharmacodynamics as well as relative and absolute contraindications, and offers alternatives and options. The user can override the program's recommendations and can readily access brief or detailed prescribing information, clinical practice guidelines, or the medical literature. The logic of the program can be modified using a series of tables, without the need for reprogramming.
Results: A prototype system has been constructed, tested with real and synthetic data, and found to perform well. The program analyzes SMBG data, identifies problems (hypoglycemia, hyperglycemia, variability, or insufficient glucose data) and recommends adjustment of dosages, addition or discontinuation of medications. The program provides caveats appropriate to each case. Extensive safety testing has been performed. The recommendations of the program are consistent with the judgment of highly experienced endocrinologists in a large series of test cases. The software has been integrated with a Comprehensive Diabetes Management Program that provides reminders and alerts and interfaces with an electronic medical record. The program can also operate in a stand alone mode with manual entry of laboratory data and medication history.
Conclusion: This study demonstrates that computer assisted clinical decision support is feasible. The logic of the program can be custom tailored to the preferences of individual clinics and physicians.
Supported by: TATRC

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Acceptance and outcome of knowledge-based decision support in routine diabetes care is strongly related to HbA_{1c} at baseline
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Background and aims: The Diabetiva© program launched 2006 by the German health insurance fund BBK Gesundheit offers continuous glucose monitoring (CGM) and decision support generated by the Karlsburg diabetes management system KADIS® to their insured diabetics. Diabetiva© is open for diabetics with cardiovascular risk and focuses on improvement of routine out-patient diabetes care according to the guidelines of the German Diabetes Association. We addressed the question, whether acceptance of decision support and metabolic outcome differs between general practitioners (GP) and diabetes specialists (DSP) involved in the Diabetiva© program.

Materials and methods: The Diabetiva© timeline includes an annual CGM followed by decision support for therapy optimization and quarterly medical check-up including HbA_{1c} detection. Patients with two CGM readings (*n*=352) were analyzed retrospectively for acceptance of the KADIS°-based decision support by the GP or DSP using a questionnaire and the outcome of the Diabetiva© program, with HbA_{1c} as primary outcome parameter.
Results: After running Diabetiva° for 36 months 799 insured diabetics (95.9% Type 2 diabetes) were enrolled and had received 1,354 CGMs. Patients were cared for by 299 GPs and 44 DSPs. 352 Patients performed already two or more CGM trails and could therefore be considered for the final outcome evaluation. For these patients approximately 74% of physicians accepted KADIS° as patient-focused support to optimize diabetes therapies; 39% used the therapeutic regimes without changes and 35 % used slight modifications. 26% of physicians did not accept KADIS°-based decision support. Logistic regression revealed that KADIS° acceptance was significantly depended on HbA1c at baseline (p<0.05). GP or DSP and type of therapy had no influence whether on acceptance nor on outcome parameters. Multiple regression analysis revealed that HbA1c and secondary outcome parameters 24 months after enrolment into Diabetiva© depend only from acceptance of KADIS° and from HbA1c at baseline (p<0.001). Again, GP or DSP type of therapy, age, onset of diabetes, BMI, and gender had no significant influence on the outcome parameters. If KADIS° was accepted HbA1c could be decreased overall by -0.38 ± 0.69% (p<0.01), whereas HbA1c at baseline <6.5% HbA1c increased by +0.05 ± 0.39% (p<0.01) abd decreased at 6.5 - 7.0% by -0.22% ± 0.49% (p< 0.01); at 7.0 to 7.5% by -0.42 ± 0.52% (p< 0.01); at 7.5 to 8.0% by -0.52 ± 0.0.44% (p<0.01); and at >8.0% by -1.31 ± 0.79%. But if KADIS°-based decision support was declined the impact of Diabetiva© was completely diminished: overall increase of HbA1c by +0.40 ± 0.77% (p<0.01).
Conclusion: Decision support is highly accepted by GPs as well as DSPs especially for diabetics with elevated HbA1c at baseline. The high acceptance rate of 74% and the significant decrease in HbA1c reveal that KADIS° in combination with CGM is an useful tool to support effectively outpatient management in routine diabetes care.

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Self-management support - a comparison of current practices for diabetes care in the Danish Healthcare System and Kaiser Permanente

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Background and aims: Self-management support is considered an essential part of care for people with diabetes. Despite the availability of internationally-accepted treatment guidelines describing optimal management of patients with diabetes many patients do not receive such level of care and support. The aim of this study was to investigate receipt of self-management support (SMS), and self-management behaviours of patients with diabetes in two healthcare settings: Kaiser Permanente, Northern California (KPNC) and the Danish Healthcare System (DHS).
Materials and methods: Using self-administered questionnaires administered in 2006 and 2007, 1871 diabetic patients (DHS=1548 subjects, 75% response rate; KPNC=323, 61% response rate) reported on the amount of SMS received during the past year. Using logistic regression approaches, we compared the percentages of patients reporting any receipt of each type of SMS and SM behaviours.
Results: Receipt of SMS varied substantially between the two systems. Diabetic patients in KPNC more frequently reported receiving all types of SMS for their disease than did patients in DHS: among KPNC and DHS patients respectively, 85% vs. 72% (p<0.0001) discussed methods to prevent disease deterioration with their doctor; 77% vs. 58% (P<0.0001) received support to set individual disease control goals; 73% vs. 47% (P<0.0001) engaged in making plans for their treatment; 59% vs. 26% (P<0.0001) had adverse effects of the medication prescribed to them explained; and 72% vs. 62% (p<0.005) experienced shared decision making. Substantially fewer patients in both systems used tools and approaches to support SM, though more patients in KPNC reported any use compared to DHS patients: 34% vs. 11% (P<0.0001) used existing patient education programs or support groups; 38% vs. 28% (P<0.0005) used websites with information about health and illness; and 56% vs. 34% (P<0.0001) used written materials about managing their health condition. Less than half of the respondents in both systems reported that they took their diabetes medication as prescribed and followed the national guidelines for exercise.
Conclusion: While patient self-management is associated with better patient outcomes, not all patients receive support from their physicians in these efforts. We found substantial differences in the frequency of SMS received across the two health care systems. For all aspects of SMS KP performed better that the DHS. Patient self-management represents an important but under-supported area of care for those with chronic conditions. Efforts to improve SMS could help address quality concerns in both Denmark and the United States.
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Type 2 diabetes treatment information for patients in the Internet. Is it useful for shared decision making?

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Internet is widely used by patients to retrieve updated health information. Consumer oriented treatment information should facilitate shared decision making between patients and health providers. The DISCERN tool has been developed and validated to evaluate quality of information about treatment choices. It scores 15 different items plus a global score from 1 (poor) to 5 (excellent). To facilitate shared decision making, uncertainty topics should be covered, and readability should be adequate.
Objective: To evaluate the quality of the information about treatment choices for type-2 diabetes available in consumer oriented websites using the DISCERN tool. To evaluate readability of consumer oriented web pages about diabetes treatments.
Materials and methods: The first thirty websites obtained from Google, Yahoo and MSN search engines using the terms "type 2 diabetes" and "treatment" were retrieved. Websites were selected by one investigator according to pre-specified inclusion and exclusion criteria. Non-selected websites were additionally reviewed by a second investigator to confirm the appropriateness of its non-selection. Selected websites were evaluated by a third investigator using the DISCERN instrument adapted to type-2 diabetes treatments. Two random samples of websites were evaluated by two additional investigators to evaluate the agreement of the evaluation between investigators using the weighted kappa statistic. Readability was evaluated using the Flesch Reading Ease and the Flesch-Kinkaid Grade Level scores obtained using a Microsoft Word processor. Additionally we evaluated the presence of any reference to the DCCT, UKPDS, ACCORD, ADVANCE and VADT studies.
Results: After the selection process 37 websites were finally evaluated. The main reason for exclusion was being retrieved in various search engines. Sixty-five percent of websites scored 3 or more in the global item indicating a moderate to excellent global information quality. More than 50% of websites scored less than 3 in the items "addressing uncertainty areas" (59%), "sources of information" (54%) and "benefits of treatments" (51%). We could not find any reference to the DCCT or UKPDS studies in 46% of websites and to the ACCORD, ADVANCE or VADT studies in 81% of them. Flesch Reading Ease was 44±13.2 (mean±SD) and Flesch-Kinkaid Grade Level 10.6±1.8 (mean±SD).
Conclusion: Although the global quality of diabetes information in the Internet seems adequate, flaws still exist in items of special interest for shared decision making about type-2 diabetes treatment choices such as uncertainty and benefits of diabetes treatments. The information available shows a tendency to more frequently cite studies with positive results instead of their counterparts. Readability scores of the evaluated websites are below the readability recommended for information addressed to the general population.

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Telemedicine support using the DIABEO software on a smartphone improves HbA_{1c} in poorly controlled type 1 diabetic patients: the randomised, 6-month, multicenter TeleDiab-1 trial

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Background and aims: Diabeo software uploaded to a smartphone can determine the appropriate prandial insulin dose and basal adjustments for the patient, using personally tailored algorithms. Data transmission then facilitates follow-up through teleconsultations. We assessed the efficacy on HbA1c of the home use of Diabeo by chronically poorly controlled type 1 diabetic (T1D) patients.
Materials and methods: Adult patients (n=180) with T1D (> 1 year), using a basal-bolus insulin regimen (> 6 months), with HbA1c ≥ 8%, were randomized to a continuation of usual quarterly follow-up (G1), the home use of Diabeo on a smartphone suggesting insulin doses with quarterly visits (G2) or the similar use of Diabeo associated with phone calls every 2 weeks but no visit (G3) for 6 months.
Results: Six month HbA1c in G3 (8.41% ± 1.04%) was lower than in G1 (9.10% ± 1.16%; p = 0.0019). G2 displayed intermediate results (8.63 % ± 1.07%). HbA1c decreased from baseline by 0.49% ± 0.89% (p<0.001) in G2 and 0.73% ± 0.84 (p<0.001) in G3; no improvement was seen for G1 (+0.18% ± 0.93%). HbA1c improvement in G3 compared to G1 was 0.91% [0.60; 1.21] (improvement in G2: 0.67% [0.35; 0.99])). There was no difference in the frequency of hypoglycemia or in medical time spent for hospital or telephone consultations. However, patients in G1 and G2 spent nearly five hours more than G3 patients attending hospital visits.
Conclusion: The Diabeo system allows a substantial improvement in HbA1c in poorly controlled T1D patients without requiring more medical time than usual care.
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Patient evaluation of education using US Diabetes Conversation Maps™ in the IDEA Study

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Background and aims: In an effort to improve self-efficacy and clinical outcomes, a new international approach to diabetes education using Conversation Maps™ (CM) has emerged. We are conducting a randomized trial to evaluate the effectiveness of this interactive, group-based learning experience using CM, which we call IDEA (Interactive Dialogue to Educate and Activate). In addition to clinical and behavioural outcomes, it is important to evaluate patient satisfaction with the experience of this novel, group-based learning method in comparison to conventional one-on-one diabetes education. The objective of this analysis is to report the data collected on the experience of patients who participated in the group and individual educational arms of the IDEA study.
Methods: 623 consented subjects with pre-existing type 2 diabetes and an A1c>=7% were randomized to group or individual education or to usual care (no education). Subjects receiving education were asked to complete a standardized evaluation regarding the subject content and the educator after each educational session. Evaluation forms contained no personally identifiable information, and included a sealable return envelope pre-addressed to the study coordinator. They were made up of likert scale questions with responses from 1-5, with 5 being the most affirmative. Overall evaluation scores were the sum of responses scaled to 100 for the content, educator, and group-specific evaluation questions. Means and standard deviations were computed for overall scores. Patient experience in each treatment arm was compared by t-tests. Relationships of demographic, psychosocial and behavioural measures with patient experience were estimated by correlations with overall evaluation scores stratified by treatment arm.
Results: Of the 489 patients attending the educational sessions, with a mean A1c of 8.2 and age 62, evaluations were collected from 87% of GE and 93% of IE participants. Both GE and IE were rated high, but overall educator ratings were slightly higher for IE, mean(SD) for GE 90.3(8.7) and IE 94.6(7.3), (p<0.0001). No significant difference was found between GE and IE for overall content ratings. No associations were found between evaluation scores and completion of the intervention in either education arm. No significant correlations were found between evaluation scores and baseline depression, self-care profiles or quality of life. Extroverted personality was weakly correlated with a more positive group experience. Diabetes empowerment was weakly correlated with favorable content and educator ratings in IE and favorable content and group experience ratings in the GE arm.
Conclusion: Overall, both education arms were perceived quite positively by patients, though IE was rated slightly higher than GE. However, higher evaluations scores were not associated with intervention completion rates. More empowered patients tended to rate both methods of education higher. Not surprisingly, extroversion was positively correlated with more positive group experiences. These findings support the opinions we heard from educators that more outgoing patients particularly liked the group experience. Forthcoming clinical, emotional, and behavioural outcomes of IDEA educational interventions will be very interesting and important.
Supported by: Merck and Co., Inc.

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Carelink, Skype and Facebook improve diabetes control in adolescents on pump therapy

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Background and aims: To report results from Carelink, Skype and Facebook as tools to improve diabetes control in diabetic adolescents on Medtronic PRT (insulin pump with glucose sensor).
Materials and methods: A total of 38 adolescents with type 1 diabetes, ages 13-22, were randomized in to groups: Regular visits (Group 1)-as standard medical protocol with regular visits at clinic, where data was downloaded at the clinic and intervention (pump settings-basal bolus insulin, education) were given to the patient and Internet visits (Group 2)- as protocol using Carelink personal program (Medtronic Diabetes), where the data was downloaded by the patient at home and interventions (same as group 1) were given via Skype (sound and video) and Facebook (written reports and chats). A1C was obtained before, three and six months after the study.
Results: Regular visits were 11.2±1.2 patients in group 1 and Internet visits were 12.8±2.4 per patient in group 2 retrospectively. There was significantly improvement in both groups (group 1 and 2 retrospectively, 7.4 ±0.9% and 7.5±1.1% on beginning with 6.2±0.8 % and 6.3±1.0%, p<0.05). Internet visits were more preferable by the patients.
Conclusion: This brief trial suggests that adolescents with type 1 diabetes prefer to make contact with their health care providers via internet, where new technologies using specific software like Carelink, Skype and Facebook can improve diabetes control same as regular clinic visits.

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Structured blood glucose monitoring intervention leads to significant glycaemic improvement in poorly controlled, non-insulin treated type 2 diabetes: results from the STeP study

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Background and aims: While blood glucose monitoring (SMBG) is known to be beneficial among insulin users, its value and utility in insulin-naïve type 2 diabetes (T2DM) remains uncertain. The Structured Testing Protocol (STeP) study assessed the effectiveness of structured SMBG use in poorly-controlled, insulin-naïve T2DM subjects.
Materials and methods: This 1-year, prospective, cluster-randomized, multicenter, clinical trial recruited 522 poorly-controlled (HbA1c ≥7.5%), insulin-naïve T2DM subjects from 35 US primary care practices. Subjects were randomized to a structured testing protocol (STG) or active control (ACG). STG subjects used the Accu-Chek® 360° View Blood Glucose Analysis System, an easy-to-use paper tool that facilitates collection and interpretation of 7-point glucose profiles over 3 consecutive days. STG subjects completed the tool on a quarterly basis and brought it to medical visits. All STG subjects received standardized instruction in SMBG, pattern recognition and interpretation. STG physicians received an algorithm for suggested medication strategies in response to observed SMBG patterns. All STG and ACG subjects received free blood glucose meters and test strips.
Results: Intent-to-treat (ITT) analysis revealed that STG subjects evidenced significantly greater mean improvement in HbA1c than ACG subjects over the 12 months (-1.2% vs. -0.9%; Δ=-0.3%; p=0.04). Unlike previous studies, there was a high degree of protocol adherence: 70% of STG subjects who completed the study recorded ≥80% of all SMBG measurements at ≥4 of the 5 quarterly protocol visits. Per protocol analysis revealed an even greater HbA1c reduction in STG vs. ACG subjects than was seen in the ITT analysis (-1.3% vs. -0.8%; Δ=-0.5%; p<0.003). Examination of STG subjects' 7-point profiles revealed significant reductions from baseline in glucose levels at all pre- and postprandial time points (p<0.01) and in MAGE (p=0.005). Subgroup analysis of HbA1c changes over the 12 months indicated that STG subjects who reported no SMBG at baseline profited more from the intervention than those who had been previously using SMBG (-1.6% vs. -0.9%; Δ=-0.7%; p<0.0001). Similar benefits were seen among STG subjects who were taking ≤1 oral hypoglycemic agent (OHA) compared with those taking >1 OHA (-1.5% vs. -0.9%; Δ=-0.6%; p<0.0001).
Conclusion: Structured SMBG promotes significantly better glycemic control over time in non-insulin-treated T2DM when both patients and physicians collaborate in the collection, interpretation and appropriate utilization of SMBG. Approximately two-thirds of patients adhered well to the treatment protocol, suggesting that structured quarterly testing is practical as well as beneficial. Structured SMBG may be of even greater benefit in those patients with little previous history of SMBG use and among those on relatively few OHAs at baseline.

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Influence of glucose self-monitoring on glycaemic control in patients with type 2 diabetes mellitus

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Background and aims: The aim of study is to assess if glucose monitoring patients with diabetes mellitus type 2 (DM2) is associated with better glucose control in different groups of patients being observed in primary health care settings.
Materials and methods: Data have been collected as part of mobile diabetic center program in patients with DM2 observed in primary health care. Glucose self-monitoring was assessed by questioning the patient about use of glucometer, usual frequency of glucose self-measurement and glucose levels in the week before visit. Patients measuring glucose at least once a week were referred to as performing glucose self-measurement. Glycemic control was determined by HbA1c level. Data are presented in M(SD) format, comparison of groups is done with Mann-Whitney U-criteria.
Results: 235 patients with DM2 have been examined with average age of 59(9.0) years and duration of diabetes of 9(6.7) years. 79% of patients were female. Level of HbA1c was 7.8(1.9)%. Peripheral neuropathy has been detected in 88% of patients, retinopathy in 44% and nephropathy in 53%. Treatment of diabetes included sulfonurea drugs in 52%, metformin in 63% and insulin in 31% of cases (combined use of several drugs possible). 32% of patients performed glucose self-measurement. No statistically significant difference was observed in level of HbA1c between patients with and without glucose self-monitoring (7.6(1.58) vs. 7.9(2.01)% p=0.48) as well as between those who did and did not perform measurement of postprandial glucose levels (7.6(1.63)% vs. 7.5(1.52)% p = 0.47). Among patients who were treated with insulin and with duration of insulin treatment more than 1 year HbA1c was significantly lower if they performed glucose self-measurement (8.3(1.32) vs. 9.3(2.0)% p = 0.035). Also lower HbA1c level (9.1(0.82) vs. 9.8(1.42)% p = 0.007) was present in glucose self-measurement group in patients with HbA1c>8%. Levels of glucose reported by patients correlated with HbA1c level (Spearman R = 0.59 for average glucose level, p < 0.0001).
Conclusion: Glucose self-monitoring improves effectiveness of insulin treatment in patients with DM2 if patients have some experience in using insulin. This confirms necessity of more frequent glucose monitoring in this group and can be explained by maximal flexibility of insulin therapy. Among decompensated patients with DM2 there is better glucose control in people using glucose self-measurement due to lower number of persons with severe decompensation (there were no patients with HbA1c >12% among self-measurement group while such levels were observed in several patients without self-monitoring). Here results of glucose measurement may become warning signs and motivate patients and doctors for change in treatment therefore reducing time and severity of decompensation. At the same time among patients with moderate decompensation glucose measurements may be not so convincing evidence of poor treatment and therefore do not influence level of compensation. Also there can be significant group of patients with DM2 in whom good compensation can be achieved without need for complex changes in treatment and lifestyle and in whom glucose self-measurement will not affect results of treatment. These factors explain absence of difference of HbA1c depending on glucose monitoring in patients with DM2 without marked decompensation.

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ACT: Actions with the CONTOUR blood glucose meter and behaviours in frequent testers

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Background and aims: Self monitoring of blood glucose (SMBG) is a self-management tool for patients with diabetes. Features on blood glucose (BG) meters, such as meal markers for pre- and post-prandial BG levels and reminders for post-prandial testing, may prompt more focused self management, especially around mealtimes. This 6-month randomized, multicenter study evaluated if use of a BG meter (Bayer's CONTOUR) with meal marker + audible reminder and diabetes education maintains or increases the frequency of post-prandial testing in frequent testers compared to diabetes education and standard meter features alone. The impact of the 2 trial conditions on patients' SMBG information, motivation, and behavioral skill, and via changes in these parameters, on SMBG practice and decision-making was evaluated.
Materials and methods: Subjects (n = 211) had type 1 (n = 120) or type 2 (n = 90) diabetes, used meal-time insulin at least 1 meal per day and tested their BG levels at least 3 times per day. Subjects received diabetes education and were randomized to Basic (no meal marker or reminder) or Advanced (meal marker + reminder) and were instructed to record BG levels in their

logbook. Subjects were seen at baseline, 6 weeks, 3 months, and 6 months with no mandated actions between visits. Baseline testing frequency was self-reported, and meters were downloaded at visit 2-4.
Results: For the primary endpoint of frequency of post-prandial testing, the Advanced testing group had significantly more frequent post-prandial tests per week (Table 1) and significantly more paired pre- and post-prandial tests than the Basic group at each follow-up.

Table 1. Post-prandial Weekly Tests

	Visit 2 6 wks	Visit 3 12 wks	Visit 4 24 wks	V3-V2	V4-V3
Basic	9.2	7.9	7.1	P <0.01	P = 0.07
Advanced	13	12	10.2	P = 0.01	P = 0.001
P values (Basic vs Advanced)	<0.001	<0.001	<0.001		

Both groups had significant declines in A1c values (Basic 8.3 to 7.9 and Advanced 8.0 to 7.8). Correlation of changes in SMBG information, motivation, and behavioral skills as they relate to changes in SMBG frequency and understanding of results, as well as markers of glycemic control, including A1c, are seen in both type 1 and type 2 diabetes.
Conclusion: Current findings demonstrate that a meter with a meal marker + audible reminder increases post-prandial and paired testing.
Supported by: Bayer HealthCare Diabetes Care

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Can glucose meters meet tighter accuracy requirements?
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Background and aims: The ISO Standard (ISO 15197:2003) and various CLSI guidelines are undergoing revisions that are likely to result in tightening the accuracy requirements on blood glucose monitoring systems (BGMS). We evaluated four of the latest generation of BGMS to assess the potential impact of tighter criteria on system accuracy.
Materials and methods: The following 4 BGMS, each with 3 lots of strips, were evaluated for finger blood testing at a clinic: Bayer Contour®, LifeScan OneTouch® Ultra®2 (with the new OneTouch Ultra Blue test strip), Roche Accu-Chek® Aviva and Abbott FreeStyle Freedom Lite® system (with the new GDH-FAD test strip). A total of 150 diabetic subjects were included in the study. A trained operator tested the subject's fingertip blood in duplicate with the 4 systems and 2 YSI glucose analyzers, which served as the reference. The order of testing the 4 systems was rotated after each subject. To ensure sufficient number of finger blood samples with glucose concentrations below 2.8 mmol/L (50 mg/dL) and above 22.2 mmol/L (400 mg/dL) were tested, 8 samples were modified to lower the glucose concentration below 2.8 mmol/L (50 mg/dL) and another eight samples were modified to elevate above 22.2 mmol/L (400 mg/dL). The accuracy of the YSI analyzers was validated by testing the National Institute of Standards and Technology (NIST) Standard Reference Material SRM 965b. All systems and supplies were stored, handled and operated according to the manufacturer's instructions.
Results: The blood glucose concentrations of the 150 subjects and the 16 modified samples ranged from 1.3 - 25.5 mmol/L (23 to 460 mg/dL), with a mean value of 9.7 mmol/L (175 mg/dL) and a median of 8.6 mmol/L (155 mg/dL). The hematocrits of the 150 subjects ranged from 32% to 54% (mean and median, 42%), and were within the product specifications. A total of 331 to 332 tests were performed on each BGMS with 166 blood samples from 150 patients. All 4 BGMS met the minimum acceptable accuracy required by ISO 15197:2003, with ≥95% of the individual glucose results falling within ±20% of the reference, and within ±0.83 mmol/L (15 mg/dL) at glucose concentrations <4.2 mmol/L (<75 mg/dL)**. When the accuracy criterion was tightened to ±15% of the reference, and within ±0.83 mmol/L (15 mg/dL) at glucose concentrations <5.6 mmol/L (<100 mg/dL)*, less than 95% of the Bayer Contour results met criterion. When the accuracy criterion was tightened to ±10% of the reference, and within ±0.56 mmol/L (10 mg/dL) at glucose concentrations <5.6 mmol/L (<100 mg/dL)*, less than 95% of the Bayer Contour, LifeScan OneTouch Ultra2 and Roche Accu-Chek Aviva results met criterion; only the Abbott FreeStyle Freedom Lite system met this criterion.

System	Within ±5% or ±0.28 mmol/L (±5 mg/dL)*	Within ±10% or ±0.56 mmol/L (±10 mg/dL)*	Within ±15% or ±0.83 mmol/L (±15 mg/dL)*	Within ±20% or ±0.83 mmol/L (±15 mg/dL)**
FreeStyle Freedom Lite	72.3%	95.5%	99.7%	100%
Accu-Chek Aviva	60.5%	91.9%	99.1%	100%
OneTouch Ultra2	49.2%	80.4%	95.2%	97.0%
Contour	27.2%	59.5%	85.8%	97.3%

Conclusion: Not all BGMS can meet tighter accuracy criteria. Manufacturers will need to be vigilant in improving the accuracy performance of their BGMS to be ready for the anticipated changes in accuracy standards and guidelines.

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Glucose self-measurement results and life quality in patients with type 2 diabetes mellitus
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Background and aims: Health-related quality of life (HRQL) can be differently influenced by self-measurement of glucose in patients with diabetes mellitus type 2 (DM2). Improved HRQL can be expected if it improves glucose control and is associated with reduction in diabetes complication. At the same time results of glucose measurement can become additional stress-factors if patient is decompensated and is not able to improve disease control due to lack of knowledge, training or support. Aim of current study was to assess whether patients performing glucose self-measurement are different in life quality compared to these who are not and to evaluate association between results of glucose measurement and indicators of HRQL.
Materials and methods: HRQL was assessed with use of SF-12 v.2 questionnaire in patients with DM2 referred for examination in mobile diabetic center in local areas of region by internists and endocrinologists in primary healthcare. Patients have been asked about use of glucometers and if present - about usual frequency of glucose measurement and levels of glucose during a week before visit. These who checked glucose levels at least once a week were referred to as performing glucose self-measurement. Maximal, minimal and average glucose levels have been used for further assessment. HbA1c level was measured at the visit for evaluation of glycemic control. Data are presented in M(SD) format unless specified otherwise. Mann-Whitney U-criteria has been used for comparison of groups and Spearman R-criteria to find out correlations.
Results: Data were received about 104 patients whose average age was 56(9.2) years and diabetes duration 8(6.2) years. 84% of patients were female. 39% of patients received insulin with average insulin treatment duration of 5(3.0) years. Patients who did and did not perform glucose self-measurement had statistically significant differences only on role-physical scale of questionnaire (45(24) vs. 55(26) p = 0.025) with patients performing glucose monitoring having poorer life quality. Patients with HbA1c below 7% had better HRQL in physical functioning (51(32) vs. 38(33) p = 0.045), role physical scale (54(25) vs. 44(26) p = 0.044), social functioning (67(25) vs. 56(27) p = 0.048) and role emotional scale of SF-12 questionnaire (61(23) vs. 50(24) p = 0.013). In correlation analysis there was significant although weak negative correlation between glucose levels in self-measurement and physical functioning, role physical and bodily pain scales (R = -0.27 -0.31 and -0.25 with p = 0.02 0.012 and 0.035 correspondingly).
Conclusion: HRQL was significantly lower in patients with decompensated diabetes. This can be a result of hyperglycemia symptoms or development of diabetes complications in this group. Life quality was not convincingly connected with presence of glucose self-measurement. In patients who performed glucose self-measurement it worsened together with increase in glucose levels for aspects of physical health and pain. Symptoms of hyperglycemia and diabetes complications may also play a role here but psychological perception of high glucose levels is not to be forgotten. Many diabetic patients do complain that poor results of glucose by self-measurement worsen their psychological status and this may affect results of HRQL evaluation.

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Association between HbA_{1c} and self-monitoring blood glucose values in patients with type 2 diabetes

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Background and aims: The role of self-monitoring of blood glucose (SMBG) in Type 2 diabetes (T2D) management is not adequately established. Aim of the present study was to assess the relationship between HbA_{1c} and SMBG values in T2D patients, as well as determine SMBG values that define a satisfactory glycaemic control.
Materials and methods: A total of 1,000 consecutive T2D patients were examined in 3 outpatient Diabetes centers. SMBG values of the previous week were recorded as well as the HbA_{1c} value at the index visit.
Results: A total of 926 patients reported they were performing SMBG at home, of which 872 had pre-prandial values and 774 also post-prandial values in their records. A very strong correlation was found between HbA_{1c} and both pre-prandial and post-prandial SMBG values ($r=0.649$, $p<0.001$ and $r=0.641$, $p<0.001$, respectively). The correlation between HbA_{1c} and the total SMBG values (both pre-prandial and post-prandial) was even stronger ($r=0.706$, $p<0.001$). In a multivariate analysis, pre- and post-prandial SMBG values had an independent association with HbA_{1c}. According to these associations, the target value of HbA_{1c}=7.0% corresponded to a mean pre-prandial SMBG value of 120 mg/dl and to a mean 2-hour post-prandial SMBG value of 151 mg/dl.
Conclusion: In patients with T2D, SMBG values for 1 week satisfactorily define glycaemic control. Post-prandial values offer additional data for assessing diabetic control. Pre-prandial values <120 mg/dl and 2-hour post-prandial values <151 mg/dl correspond to HbA_{1c} values <7.0%.

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Structured blood glucose monitoring reduces distress and depression, and enhances well-being in poorly controlled, non-insulin treated type 2 diabetes: results from the STeP Study

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Background and aims: Recent reports have suggested that the promotion of SMBG among non-insulin treated patients with T2DM is associated with more depressive symptoms. In the Structured Testing Protocol (STeP) study, we investigated the impact of a structured SMBG intervention on diabetes distress, clinical depression and well-being over 12 months.
Materials and methods: In this prospective, cluster-randomized, multi-centered clinical trial with 522 randomly assigned, poorly-controlled (HbA1c ≥7.5%), insulin-naïve T2DM patients, we showed that patients in a structured testing SMBG group (STG) displayed larger reductions in HbA1c over 12 months than patients in an active control group (ACG). STG subjects used the Accu-Chek® 360° View Blood Glucose Analysis System, an easy-to-use paper tool that facilitates collection and interpretation of 7-point glucose profiles over 3 consecutive days. STG subjects completed the tool on a quarterly basis and brought it to medical visits. All STG subjects received standardized instruction in SMBG, pattern recognition and interpretation. STG physicians received an algorithm for suggested medication strategies in response to observed SMBG patterns. All STG and ACG subjects received free blood glucose meters and test strips. At baseline, 3, 6, 9 and 12 months, all subjects completed self-report measures to assess diabetes-specific distress (the Diabetes Distress Scale, DDS), clinical depression (the Patient Health Questionnaire 8, PHQ8), and positive well-being (the WHO5).
Results: Intent-to-treat (ITT) analyses indicated significant reductions from baseline in DDS ($p<0.001$) and PHQ8 ($p<0.001$) scores and significant increases in positive well-being (PWB) scores ($p<0.001$) for subjects in both STG and ACG at 3, 6, 9 and 12 months. STG patients who reached criteria for significant diabetes distress (mean item DDS score ≥3) and STG subjects who reached criteria for clinical depression (total PHQ8 ≥10) at baseline showed a significantly greater reduction in distress and depression over time than ACG subjects at 9 and 12 months ($p<0.03$ in both cases) No between-group differences were found for PWB over time.
Conclusion: Contrary to previous reports and using well-validated measures, we found that both treatment groups experienced significant reductions in diabetes distress and depression and increased PWB over time. Furthermore, among patients with elevated diabetes distress or clinical depression at baseline, the structured SMBG intervention was associated with significantly greater improvement in these conditions over time than for control subjects. In sum, these findings suggest that when both patients and physicians collaborate to gather, interpret and appropriately utilize structured SMBG data, emotional distress is alleviated, not worsened.

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An information-motivation-behavioural skills analysis in frequent testers

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Background and aims: Self monitoring of blood glucose (SMBG) is a behavioral tool for patients with diabetes. Features on blood glucose (BG) meters, such as meal markers for pre- and post-prandial BG levels and reminders for post-prandial testing, may better inform self-management decisions, especially around mealtimes. This 6 month randomized, multicenter study evaluated if use of a BG meter with meal marker + audible reminder and diabetes education maintains or increases frequency of postprandial testing in frequent testers compared to diabetes education and standard meter features alone. The impact of the two trial conditions on patients' SMBG information, motivation and behavioral skills on SMBG practice and decision-making, were evaluated from baseline to completion via an IMB survey. Clinical parameters reported previously. IMB correlation to clinical parameters are presented here.
Materials and methods: Subjects (n=211) had type 1 (n=120) or type 2 (n=90) diabetes, used meal time insulin at least 1 meal/day and tested their BG levels at least 3x/day. All subjects received diabetes education and were randomized to *Basic* (no meal marker or reminder) or *Advanced* (meal marker + reminder) and instructed to record BG levels in their logbook. Subjects were seen at baseline, 6 weeks, 3 months, and 6 months with no mandated actions between visits.
Results: Baseline A1c correlated with motivation at baseline and across the study, most strongly in the type 1 population ($r= -0.24$, $p<0.01$ Visit 1; $r= -0.22$, $p<0.05$ Visit 4). In patients with type 2, increased information correlated with increased Glycomark, a reported measure of improved post-prandial glucose control ($r=0.32$, $p<0.007$ Visit 1: $r=0.38$, $p< 0.003$ Visit 4). At completion, the Advanced group had stronger understanding and belief that food and/or exercise have an effect on BG levels and were less anxious about blood sugar testing compared to subjects who did not use this feature. In subjects with type 1 and type 2 diabetes, using the meal marker resulted in significant increase in post-prandial testing (15% Visit 1 to 50% Visit 4) as well as significant improvement in understanding pre- and post-meal results over time (33% at time 1 to 72% at time 4).
Conclusion: SMBG can be an effective self-management tool that may be instrumental in achieving glycemic control among adults with type 1 and type 2 diabetes. Understanding and utilization of particular meter features may improve the value of SMBG. Correlation of changes in SMBG information, motivation, behavioral skills as they relate to changes in SMBG frequency and understanding of results, as well as markers of glycemic control, including A1c, are seen in both type 1 and type 2 diabetes.
Supported by: BHC, BDC

PS 100 Continuous glucose monitoring systems: devices, practice and outcomes

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Prospective data evaluation of the application of a multisensor device for non invasive continuous glucose monitoring

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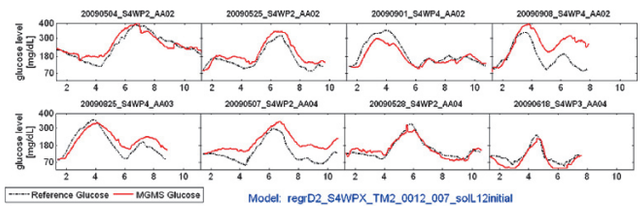
Background and aims: We have previously reported about the findings in clinical-experimental studies with a novel Multisensor system for non invasive continuous glucose monitoring. The Multisensor measures skin impedance and optical skin characteristics in several frequency bands of the electromagnetic spectrum as well as temperature, acceleration and humidity. In this study a Multisensor version with fully integrated sensors and battery in a miniaturised housing (54 x 65 x 13 mm) was experimentally tested to compare the outcome to previous findings using earlier versions.
Materials and methods: Six T1DM patients (age 44±16 y; BMI 24.1±1.3 kg/m2, duration of diabetes 27±12 y; HbA1c 7.3±1.0%) wore the same Multisensor at the upper arm. In total these patients performed 45 in-clinic study days; each patient performed on average seven study days (min. 5 and max. 10 days). A study day lasted approximately 10 hrs (min. 8 and max. 11 hrs) and glucose changes were induced by the administration of an oral or i.v. glucose solution. Blood glucose was measured for reference using a HemoCue analyser. Several prospective data evaluation routines were applied. The first 22 study day's data spanning all subjects were used to train a linear regression model. The global model derived was then prospectively applied to the data of the remaining 23 study days allowing for external validation. One initial baseline adjustment at the very beginning of each study day was used to adjust the level of the glucose estimate.
Results: Figure 1 shows the time series of all 23 externally validated study days. These profiles were obtained with fully prospective data evaluation using a global model with one initial calibration point. When comparing the estimated glucose to the blood glucose reference values, the model yielded a Mean Absolute Relative Difference (MARD) of 40.8%, a Mean Absolute Difference (MAD) of 51.9 mg/dL, and an R2 of 0.70 on average per study day. The Clarke error grid analyses showed 89.0% of paired glucose values in A+B, 4.5% in C, 4.6% in D and 1.9% in the E region.
Conclusion: This work demonstrates that glucose variations under controlled conditions can be monitored non invasively by a prospectively applied multiple regression statistical model. The findings from this study indicate that further development steps in the Multisensor system should not affect the estimation performance. In this respect, the expected model performance can be more reliably judged at the stage of clinical validation.



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Simultaneous non invasive continuous glucose monitoring on the left and right arm using two multisensor devices

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Background and aims: We have previously reported about the findings in clinical-experimental studies with a novel Multisensor system for non invasive continuous glucose monitoring. The Multisensor measures skin impedance and optical skin characteristics in several frequency bands of the electromagnetic spectrum. In this study a Multisensor version with fully integrated sensors and battery in a miniaturised housing (54 x 65 x 13 mm) was experimentally tested, investigating location related measurement characteristics.
Materials and methods: Four T1DM patients (age 43±9 y; BMI 24.5±3.7 kg/m^2, duration of diabetes 22±11 y; HbA1c 7.7±0.5%) performed 4 in-clinic study days with a Multisensor attached to the left and right upper arm. As a result, 32 datasets from 16 study days were obtained. The Multisensors were exchanged between the patients and the left and right arm according to a Graeco-Latin Square. Glycaemia was varied using 4 different glucose profiles. For each study day, one of the four different glucose profiles was induced via oral Carbohydrate loads. Blood glucose was measured for reference using a HemoCue analyser. Different data evaluation routines were applied to the Multisensor data in order to obtain global (identical coefficients) and personal (personal coefficients) models that were used for cross validation.
Results: Figure 1 shows all 32 glucose profiles obtained during the 16 study days, using the global model with a prospective initial baseline calibration (CEG A 44.9, B 48.0, C 3.6, D 3.0, E 0.5%). The following performance metrics was obtained from the different models. In each model an initial baseline calibration was used at the beginning of each study day (IB) as well as a full day baseline calibration (FB), with Average R2 = coefficient of determination on average over the study days, MAD= Mean Absolute Difference [mg/dL], MARD= Mean Absolute Relative Difference [%]. Global IB: 0.76, 47, 32.3; Global FB: 0.75, 29.9, 21.3; Personal IB: 0.85, 43.3, 30.7; Personal FB: 0.84, 24.1, 17.6.
Conclusion: The glucose time courses estimated by the Multisensors from the two arms are repeatably comparable, even with a global model with one initial baseline calibration only. This indicates that the sensor signal characteristics are robust enough to allow changing from one arm to the other using the same device settings and calibration. That represents thus a further indication that the Multisensor approach for non invasive glucose monitoring under such conditions is possible. It is also an indication that a personal model may be able to track glucose more accurately than a global model.

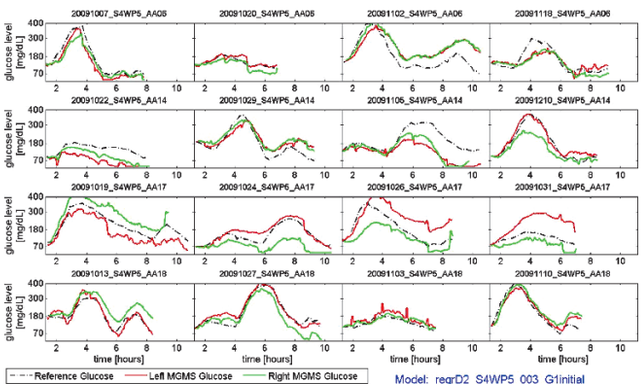

Figure 1: Overview of all 32 predicted glucose profiles with a fully prospective data evaluation of the two Multisensor devices on the left (red) and right (green) arm using a global model with one initial calibration point (Global IB).

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Measuring within-patient glycaemic variability: a best practice

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Background and aims: The role of glycaemic variability (GV) in the development of diabetes complications and hypoglycaemia in patients (pts) with diabetes remains controversial. Our aim was to compare current GV measures through simulated self-monitored blood glucose (SMBG) and continuous glucose monitoring (CGM) data points to determine which measure carries the highest statistical power and effect size.
Materials and methods: We simulated datasets for 1,000 trials with pts with type 1 (20%) and type 2 (80%) diabetes. Three days of 7-point SMBG profiles (n=240 pts/trial) and 288-point CGM profiles (n=20/trial) were generated using a gamma distribution which created two patient groups, Groups 1 and 2. To assess the effect of different GV measures independent of mean BG, both groups had a mean BG of 7.94 mmol/L. GV (defined as velocity of BG change over time [mmol/L per minute]) for Group 2 ("BG Change") was 50%

greater than for Group 1 ("Reference"). Patients were randomised to diabetes type, then mealtime pattern (3 meals a day ± snack ± dawn phenomenon) and meal start time, which varied by patient-day, were also randomised. Groups were compared using a t-test for each measure. Power was calculated as the proportion of trials with a p-value <0.05. Effect size (estimate of the measure's strength to detect the difference in GV between Groups 1 and 2) was calculated by averaging the difference between groups (Group 2 - Group 1) divided by the standard deviation for the pooled groups. A Pearson's matrix was obtained for total [SD], within-day and day-to-day measures to further characterise them.

Results: See table.

Results for total GV for all measures (only SD results shown) were similar to within-day GV results. For SMBG within-day GV, SD had the highest power and effect size and for SMBG day-to-day GV, ADRR had the highest power and effect size. For CGM within-day GV, ACM had the highest power and effect size and for CGM day-to-day GV, ADRR had the highest power and effect size. For total GV, SD resulted in 100% power for both SBGM and CGM and a 2.19 and 3.14 mmol/L effect size for SMBG and CGM, respectively. For SMBG within-day, all measures correlated with each other (rho > 0.59). For SMBG day-to-day measures, CONGA and MODD highly correlated (rho = 0.73). For CGM within-day, all measures (except ARC and SD[ARC]) highly correlated with each other (rho > 0.81). For CGM day-to-day measures, ADRR, CONGA, and MODD highly correlated (rho > 0.75).

Conclusions: Effect size helped differentiate measures, and the lower power for CGM suggests more pts per trial are needed. For analysing SMBG and CGM within-patient GV data, SD is recommended for within-day and ADRR for day-to-day GV. Although ACM had a slightly higher effect size and Range and IQR had a similar effect size for CGM within-day GV, SD is preferred because it is consistent with the SMBG results. Because within-day (SMBG) and day-to-day (SMBG, CGM) measures are highly correlated, using only SD (within-day) and ADRR (day-to-day) to measure GV is currently justified.

	SMBG		CGM	
Measure	Power (%)	Effect Size	Power (%)	Effect Size
SD (mmol/L)				
Total	100	2.19	100	3.14
Within-Day	100	2.20	100	3.14
Day-to-Day	93.2	0.33	76.6	0.14
M-Value				
Within-Day	100	1.91	97.6	2.60
J-Index (mmol/L)				
Within-Day	100	2.17	99.6	2.95
MODD (mmol/L)				
Day-to-Day	100	0.66	87.0	1.99
ACM (mmol/L)				
Within-Day	100	2.17	100	3.15
ADRR				
Day-to-Day	100	1.90	100	2.77
ARC (mmol/L)				
Within-Day	100	1.94	79.0	-0.14
Within-Day	100	2.01	100	2.55
CONGA (mmol/L)				
Day-to-Day	100	0.53	86.6	2.01
Within-Day	100	1.47	85.1	1.91
Within-Day	100	2.03	99.3	2.82
IQR (mmol/L)				
Within-Day	100	1.81	100	3.13
Range (mmol/L)				
Within-Day	100	2.05	100	3.14
SD[ARC] (mmol/L)				
Within-Day	100	1.46	79.6	0.53

Abbreviations: ACM = average change from median; ADRR = average daily risk range; ARC = average rate of change; CONGA = continuous overall net glycaemic action; IQR = inter-quartile range; MODD = mean of daily differences; SD = standard deviation; SD[ARC] = standard deviation of average rate of change

Supported by: Eli Lilly and Company

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Assessment of the variance of the ambulatory glucose profile over 3 to 20 days of continuous glucose monitoring

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Background and aims: The Ambulatory Glucose Profile (AGP) has been proposed as an effective way to identify trends in glucose abnormalities in people with diabetes using continuous glucose monitoring (CGM). The aim of this study was to evaluate the minimum number of days of CGM needed to arrive at stable glucose patterns revealed by AGP analysis.

Materials and methods: AGP analysis was performed utilizing 67 adult subjects (T1DM = 47, T2DM = 20) who participated in a study that began with 20 days of masked CGM, using the FreeStyle Navigator® System. Subjects were not able to see their CGM glucose values or trends and did not have glucose threshold or projected alarms available. Only masked data were evaluated in order to minimize effects of therapy adjustments on the evaluation. Statistics for each of 3 to 19 days of CGM data were compared to the 20-day values and evaluated on a per-subject basis. For overall summary statistics (mean, standard deviation, 10th, 25th, 50th, 75th, 90th percentiles, inter-quartile range, mean change in the hourly median curve), equivalence criteria of 90-110% of the 20-day value were evaluated. For overall rates of glucose above, below or within the target of 3.89-7.78 mmol/L (70-140 mg/dL), the absolute difference compared to the 20-day value was evaluated at equivalence criteria (based on scaling the standard error of the overall mean) of 6.40%, 1.45%, and 5.46%, respectively. For hourly AGP percentile lines (10th, 25th, 50th, 75th, 90th) the mean absolute relative difference compared to the corresponding 20-day line was calculated and evaluated against the equivalence criteria <10%.

Results: A summary of the 20-day statistics and the relationship between the number of days needed for an AGP statistic to meet the equivalence criteria for 70%, 80% and 90% of subjects is shown in Table 1. After 10 days, the glucose mean, standard deviation, 50th, 75th, and 90th percentiles are within 10% of the 20-day value for more than 80% of subjects. After 14 days, the rates of above, below and within target, the 10th and 25th percentiles, and the hourly AGP percentile lines met the equivalence criteria for over 80% of patients. The interquartile range and mean change in the hourly median curve needed more days of CGM to approximate the 20-day value for 80% of subjects: 15 and 18 days, respectively.

Conclusion: AGP analysis promises to be an effective tool for identifying glucose abnormalities and may allow the use of evidence-based and protocol-driven medical practices to select appropriate therapies to address those abnormalities. Clinical evidence is lacking to support the assumption used in this study that AGP analysis of 20 days of CGM can identify clinically important glucose trends and patterns. Within that context, however, this analysis suggests that a minimum of 14 days of CGM provides identification of individual glucose patterns. Prospective studies are needed to provide clinical evidence establishing the benefits of identifying patterns with AGP analsyis that affect treatment and improve outcomes.

Table 1. Summary of 20-day AGP statistics and number of CGM days needed to reach equivalence.

Ambulatory Glucose Profile Statistic	Mean (SD) [Min, Max] of 20-day Values for Subjects	Criteria for Equivalence with 20-day Statistic	Number of Days for % of Subjects to Meet Equivalence Criteria		
			70%	80%	90%
Mean	9.6 (1.8) [6.3, 13.6] mmol/L	ARD < 10%	6	9	11
Standard Deviation	3.4 (0.8) [1.9, 5.4] mmol/L	ARD < 10%	8	10	15
10th percentile	5.7 (1.3) [3.4, 8.5] mmol/L	ARD < 10%	8	12	15
25th percentile	7.3 (1.6) [4.6, 10.8] mmol/L	ARD < 10%	8	11	15
50th percentile	9.4 (1.8) [6.1, 13.5] mmol/L	ARD < 10%	7	9	12
75th percentile	11.7 (2.2) [7.3, 17] mmol/L	ARD < 10%	6	9	12
90th percentile	13.9 (2.5) [8.7, 21.2] mmol/L	ARD < 10%	7	9	13
Inter-Quartile Range	4.4 (1.2) [2.2, 7.7] mmol/L	ARD < 10%	13	15	16
Mean Change in Median Curve	0.6 (0.2) [0.2, 1.2] (mmol/L)/h	ARD < 10%	18	18	-
% Above 7.78 mmol/L	63 (18.1) [19, 92] %	AD < 6.40%	10	13	15
% Within 3.89 - 7.78 mmol/L	33 (15.4) [8, 74] %	AD < 5.46%	11	14	15
% Below 3.87 mmol/L	4.2 (4.1) [0, 15.6] %	AD < 1.45%	9	14	15
Hourly 10th Percentile Curve	-	MARD < 10%	10	13	15
Hourly 25th Percentile Curve	-	MARD < 10%	10	12	15
Hourly 50th Percentile Curve	-	MARD < 10%	11	13	14
Hourly 75th Percentile Curve	-	MARD < 10%	12	14	16
Hourly 90th Percentile Curve	-	MARD < 10%	13	14	16

ARD = Absolute Relative Difference
AD = Absolute Difference
MARD = Mean Absolute Relative Difference

Supported by: Abbott Diabetes Care

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Assessment of postprandial glucose control; a consideration from continuous glucose monitoring

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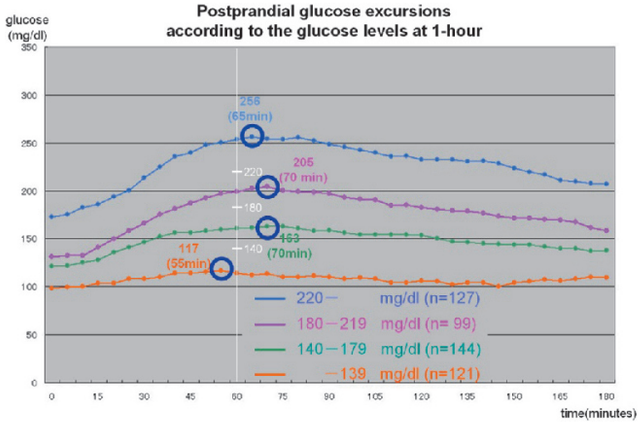
Background and aims: Importance of postprandial glucose control has been appreciated, although the consensus on its assessment is not established. This study was undertaken to reveal characteristics of postprandial glucose control and make a consideration on its assessment.
Materials and methods: Glucose levels until 3-hrs postprandial were measured with Continuous Glucose Monitor (CGM) in a total of 491 meals. These were obtained from a total of 103 diabetic patients (41 with type 1, 56 with type 2, and 6 with other types; with ages of 52.6±13.7years, BMI 23.0±3.9kg/m2, and HbA1c 9.1±2.2%, mean±SD). Eighty-one patients were treated with insulin (mostly with multiple injections or CSII), 11 with oral hypoglycemic agents, and 11 with diet therapy alone. Most of the patients were admitted and given controlled diet comprising 50% of energy intake as carbohydrate.
Results: Median glucose levels of the 491 meals were 126 (pre-prandial), 155 (at 30 min postprandial), 175 (at 60), 172 (at 90), 166 (at 120), 156 (at 150), and 149 mg/dl (at 180), respectively. Maximal median value was 176 mg/dl at 65 min. Pre-prandial and maximal postprandial glucose levels after breakfast, lunch, and supper were 130 and 191 (at 60), 127 and 163 (at 75), 135 and 186 (at 80), respectively. Postprandial glucose levels 45~130 min after lunch were significantly lower than those after breakfast and supper, suggesting presence of second meal effect. Between patients with or without insulin therapy, glucose excursions after meal were essentially analogous and both glucose peaks were at around 60 min. When the subjects were divided into 4 groups according to pre-prandial glucose levels (-109, 110-129, 130-159, and 160- mg/dl), corresponding maximal postprandial glucose levels were 150 (at 95), 165 (at 75), 183 (at 80), and 232 mg/dl (at 60), respectively. There was a trend that a glucose peak appears earlier as pre-prandial glucose levels increase. When the subjects were divided into 4 groups according to glucose levels at 2-hours postprandial (-140, 140-179, 180-219, and 220- mg/dl), corresponding maximal postprandial glucose levels were 133 (at 45), 173 (at 65), 205 (at 65), and 262 mg/dl (at 105), respectively. There was a trend that a glucose peak appears later as 2-hours postprandial glucose levels increase. Then we divided the subjects into 4 groups according to glucose levels at 1-hour postprandial by using the same criteria at 2-hours. Corresponding maximal postprandial glucose levels were 117 (at 55), 163 (at 70), 205 (at 70), and 256 mg/dl (at 65), respectively. The glucose peaks were observed at around 60 min, irrespective of glucose control (Figure).
Conclusion: When postprandial glucose levels are assessed at 2-hours, "real" glucose peaks are observed at 45~105 min depending on glucose control. Given the glucose spike plays an important role on vascular complications, assessment at 1-hour would be appreciated since the glucose peaks are captured at this point irrespective of glucose control.



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Predictors of continuous glucose monitoring (CGM) variability and associations with patient satisfaction and health perceptions in insulin-treated diabetes

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Background and aims: Glycemic variability is typically estimated using 7-point glucose profiles, which might not be sensitive to clinical predictors and patient-centered health outcomes. We modeled continuous glucose monitor (CGM) variability to determine if insulin regimen, type of diabetes, age, sex, BMI and HbA1c predict CGM within-day standard deviations (SD), and if SD changes are associated with patient satisfaction (PS) and perceived health (PH).
Materials and methods: We analyzed CGM data from 306 insulin-treated T2DM and 82 T1DM (47% male, age 54±11 yrs, HbA1c 7.8±0.7%) who were randomized to open-label daily insulin glargine + premeal glulisine (GG; n=192) or BID analogue premix 75/25 or 70/30 (PM; n=196) for 12 wks (P1), and then crossed over to the alternate treatment for 12 wks (P2). Patients were contacted weekly to ensure compliance with a titration algorithm with a target HbA1c < 7.0%. Three-day CGM and HbA1c were obtained at Wks 0, 12 and 24. Patients completed clinic-based PS and PH questionnaires at Wks 0, 8, 12, 20 and 24. CGM estimates were obtained for each patient from the 3-day session (288 glucoses/day), and regression used to model independent variables. PS was represented by the net benefit composite scale, which included 4 subscales of advocacy, general satisfaction, glycemic effectiveness and preference. The PH scale included 3 subscales of health status, vitality and sleep quality.
Results: During P1, reductions from baseline for glycemic measures were larger for GG vs PM (*p<0.001; see Table), except for % time < 3.9 mmol/l. P2 cross-over results (not shown) were similar to the between-group differences in P1. Baseline-adjusted PS (60.5±1.2 vs 45.4±1.2) and PH (427±3 vs 418±3) were higher for GG compared to PM during P1 and P2 (both p<0.01). CGM SD decreased with GG (-0.1±0.06 mmol/l, p=0.037), increased with T1DM (0.6±0.09 mmol/l), decreased by 0.02±0.004 and 0.01±0.003 mmol/l per unit increase in BMI and age, and increased by 0.4±0.04 mmol/l per unit increase in HbA1c (all p<0.001). Sex was not a significant predictor of CGM SD. The % time < 3.9 mmol/l was higher by 6.8±1.0% for T1DM vs T2DM, p<0.001. Improvement in PS was independently associated with decreases in HbA1c, CGM BG and SD (all p<0.015). PH improved with decreased CGM BG, SD and % time > 7.8 mmol/l (all p<0.05).

Table 1. Glycemic Control and Variability During Period 1 Treatment

Period 1	GG Baseline	GG Wk 12	PM Baseline	PM Wk 12
HbA1c (%)	7.8±0.05	7.1±0.05*	7.8±0.05	7.4±0.06
BG (mmol/l)	9.7±0.10	8.2±0.09*	9.7±0.09	9.1±0.09
SD (mmol/l)	2.7±0.04	2.4±0.05*	2.7±0.04	2.7±0.04
% time > 7.8 mmol/l	63.0±1.0	46.5 ±1.1*	63.9±1.0	55.6±1.0
% time < 3.9 mmol/l	5.3±0.4	7.4±0.4 (ns)	5.4±0.4	5.8±0.4

Conclusion: Lower CGM variability was associated with insulin glargine plus glulisine vs premix, and was predictive of improved PS and PH. Glucose variability was higher with increased HbA1c and lower among T2DM, older, and higher weight patients. CGM variability, PS, and PH are useful patient-oriented measures to evaluate the comparative effectiveness of diabetes treatments.
Supported by: sanofi-aventis

1057

Withdrawn

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Continuous glucose monitoring: effect on glucose control and treatment satisfaction in diabetes mellitus type 1

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Background: The effect of a continuous glucose monitoring system (CGMS) on glucose control in patients with diabetes type 1 has been explored in several studies. These have reached different conclusions, and the value of a CGMS used for short periods of time has yet to be determined.
Material and methods: In a randomized controlled cross-over trial we assessed whether one month's use of a CGMS (Medtronic Guardian RT) lowers HbA_{1c} levels and frequency of hypoglycemic episodes, compared to intensified conventional finger-prick measurements (ICFM) in patients with diabetes type 1. Treatment satisfaction (DTSQ) and health status (SF-36) was also assessed. Thirty patients (mean age 34 ± 9 yrs) with moderately good glucose control (HbA_{1c} 7.0 - 10.0%) were included in the study. They were randomized to perform either ICFM or CGMS of blood glucose for one month followed by a two months wash-out (observation) period before they were crossed over to the opposite intervention. HbA_{1c} was measured both at the end of the intervention period and the wash-out period.
Results: At inclusion mean HbA_{1c} was 7.84 ± 0.94%. The mean change in HbA_{1c} was -0.23 ± 0.10% for the CGMS period and -0.24 ± 0.09% for the ICFM period (p = 0.91). The mean change in HbA_{1c} during treatment and washout periods was -0.14 ± 0.09% for the CGMS period and -0.16 ± 0.08% for the ICFM period (p = 0.86). The frequency of hypoglycemic events was 8.2 ± 1.6 during CGMS period and 7.3 ± 1.4 during the ICFM period (p = 0.67). Treatment satisfaction and health status were also equal between treatments.
Discussion: This study does not support previous findings that CGMS is superior to ICFM in order to lower HbA_{1c} or reduce the burden of hypoglycemic episodes in adults. It could be argued that our results are based on a limited number of patients, evaluated over a short period of time, and in a patient population with moderate problems with glucose control and hypoglycemic episodes. However, we used a crossover design making the patients their own controls which strengthens the validity of our findings. The mean HbA_{1c} level was fairly low at inclusion. This may have contributed to the small decline in HbA_{1c} levels during intervention and the absence of a superior effect of GCMS in achieving good blood glucose control with less hypoglycemic events. CGMS compared to ICFM did not improve treatment satisfaction. Remarks from the participants indicate that the burden of carrying an electronic device outweighted the benefit of real-time information on glucose levels.
Conclusion: In conclusion the present study shows that the average blood glucose, evaluated by HbA_{1c}, decreased equally in both patients performing frequent self-monitoring of blood glucose, and in patients carrying a continuous glucose monitoring system for one month. The frequency of hypoglycemic episodes and treatment satisfaction were also equal in the two intervention periods. Future studies should aim at identifying subgroups of patients that show a clear benefit of using a continuous glucose monitoring system for a short time period.
Supported by: Norwegian Association of Diabetes

PS 101 Optimising resource utilisation

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Insulin initiation in accordance to NICE guidance: audit and review of Trust practice

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Background and aims: National Institute of Clinical Excellence (NICE) in the UK most recent guidance on the management of patients with type 2 diabetes mellitus (T2DM) were published in 2008 A key recommendation of this guidance was to recommend the use of NPH insulin over basal analogue insulins. These changes were performed in the light of evidence by the committee showing no definitive advantage on the use of basal analogue insulin therapy in all patients with T2DM. From mid 2008, the diabetes specialist team in our Trust changed its insulin prescribing trends in parallel with NICE guidelines. This was following the service undergoing a review using programme budgeting marginal analysis (PBMA), a methodology that uses cost savings made from one aspect of the service and reinvest in another aspect.
Materials and methods: All patients with T2DM initiated on insulin therapy by the specialist team from February 2009 to January 2010 inclusive were included in the analysis. Data of prescribing trends and patient information was obtained from electronic diabetes record system, clinical handwritten notes and dictated letters. The decision for insulin initiation was undertaken by the specialist diabetes team for all patients. Patients were started on insulin both in community and hospital care settings. Initiation of basal therapy was in concordance with NICE insulin prescribing recommendations. Cost for insulin and savings were based on an average patient use of 40 units of insulin a day and the current market price for insulin.
Results: There were a total of 82 patients (39M, 43F, median age 65 years (IQR 55-77)) patients with T2DM who were commenced on basal insulin therapy over the study period.. There were a total of 75.6% (n=62) patients initiated on NPH therapy and 24.4% (n=20) patients commenced on insulin analogues. This was a change from the prescribing pattern of 79.1% analogue insulin and 20.9% insulin NPH during the previous year. Mean HbA1c of insulin on patients on NPH improved from (mean±SE) 10.4±0.2% to 9.1±0.3% and for analogue insulin 10.9±0.6% to 9.8±0.7%. There was no difference between Hba1c levels pre and post insulin therapy between the two groups. (Mann Whitney U test). For the NPH group, there were 20 (32.2%) patients who self reported hypoglycaemia compared to 4 (20%) patient on analogue (p=0.23, chi squared test). There were no episodes of severe hypoglycaemia where third party intervention was required over the study period. The total cost savings calculated over the period based on the change in insulin prescribing from the previous year was over £ 14,300.
Conclusion: From 2002 to 2008 in England, the number of diabetes items prescribed increased by 73.3% and the total cost has risen by 93.2%. Similarly, prescribing costs for intermediate insulin and long acting insulin has increased by 13.4% from the year 2007 to 2008. There is currently no conclusive evidence in the literature to support the use of basal analogue insulin in improving mortality, morbidity or quality of life in all treated patients over the NPH insulin. The selection of patients continuing to be initiated on insulin analogues introduced a bias into the review, in that they were more likely to be residing in institutional care, therefore our data does not suggest a difference in hypoglycaemia rates. The findings of our study illustrate the cost savings that can be achieved which could be reinvested in diabetes services, it is likely over time the cost savings are likely to increase by at least this amount on a yearly basis.

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Structured blood glucose monitoring reduces HbA_{1c} levels and annual test strip consumption in poorly controlled, non-insulin treated type 2 diabetes: results from the STeP Study

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Background and aims: Conclusions from recent systematic reviews have been inconsistent regarding the cost-effectiveness of self monitoring of blood

glucose (SMBG) in insulin-naïve type 2 diabetes (T2DM). The Structured Testing Protocol (STeP) study examined the utility of structured SMBG in comparison to enhanced usual care (which included SMBG based on USA standard of care recommendations) in this population. We hypothesized that structured SMBG would be associated with improved HbA1c outcomes without increasing test strip consumption, compared with standard SMBG use.
Materials and methods: The STeP study is a prospective, cluster-randomized, multi-center, clinical trial with 522 poorly-controlled (HbA1c ≥7.5%), insulin-naïve T2DM subjects who were assigned to a structured testing protocol (STG) or an active control (ACG). STG subjects used the Accu-Chek® 360° View Blood Glucose Analysis System, an easy-to-use paper tool that facilitates collection and interpretation of 7-point glucose profiles over 3 consecutive days. STG subjects completed the tool on a quarterly basis and brought it to medical visits. All STG subjects received standardized instruction in SMBG, pattern recognition and interpretation. STG physicians received an algorithm for suggested medication strategies in response to observed SMBG patterns. All STG and ACG subjects received free blood glucose meters and test strips. Test strip consumption was measured using electronic data uploaded from blood glucose meters in both study groups.
Results: Although both groups demonstrated significant reductions in HbA1c over 12 months, intent-to-treat analysis showed a significantly greater HbA1c reduction in the STG than in the ACG (-1.2% vs. -0.9%; Δ=-0.3%; p=0.04). SMBG frequency was negatively associated with HbA1c in both groups ($p<0.05$) over time. However, STG subjects performed significantly fewer tests/day than ACG subjects (mean = 0.9 vs. 1.2, p=0.0003) over the 12 months. Extrapolating over time, this equates to a 25% difference in annual test strip consumption between the STG (329 tests/year) and ACG (438 tests/year).
Conclusion: Structured SMBG use was associated with greater reductions in HbA1c compared with standard SMBG use, and structured SMBG use required 25% less test strip consumption on average over the 12-month period than the standard approach. Therefore, structured SMBG use may be a more cost-effective approach to improving glycemic control in poorly controlled, non-insulin treated T2DM.

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Healthcare costs of fast-acting insulin analogues vs. short-acting human insulin in combination with long-acting insulin analogues for Danish patients with type 2 diabetes

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Background and aims: The aim of this study was to compare the direct healthcare costs incurred by patients with type 2 diabetes (T2D) on a basal-bolus regimen using either fast-acting insulin analogues (Insulin Aspart, Insulin Lispro or Insulin Glulusine) or short-acting human insulin. Due to local registry regulations it was not possible to analyse brand specific data.
Materials and methods: Data were extracted from registers covering the total Danish population, and included prescription data, in- and outpatient hospital data, primary care data, and demographic variables. Patients were identified in a 1-year inclusion period (2005). Inclusion criteria were at least 2 prescriptions in the index period of a long-acting insulin analogues in combination with either fast-acting insulin analogues or short-acting human insulin with no switch to the other product during follow-up. Patients with at least one oral antidiabetic drug prescription were included as T2D, and patients with >1 prescription of fast-acting insulin analogues or short-acting human insulin were classified as basal-bolus. Individual patients from the fast-acting insulin analogues group (n=445) were matched individually with patients from the short-acting human insulin group (n=85) with respect to observable variables using propensity scores. Annual healthcare costs were extracted and analysed for a follow-up period of maximum 2 years after the inclusion date.
Results: Overall annual direct healthcare costs, including prescription medicine, amounted to €4,163 in the fast-acting insulin analogue group and €5,130 in the short-acting human insulin group. Bolus insulin treatment costs were significantly higher for patients using fast-acting insulin analogues compared to patients using short-acting human insulin (€521 vs. €445). However, these costs were offset elsewhere in the health care system. Hospital cost estimates were considerably lower for the analogue group (€1,738 vs. €2,647). Given the small sample size, bootstrapped confidence intervals showed that the cost reductions were not statistically significant.
Conclusion: Using a matched cohort technique, patients with T2D on a basal-bolus regimen and fast-acting analogues are not more costly to the Danish healthcare system than patients using short-acting human insulin despite higher insulin costs. The register-based study is ongoing and is expected to be updated with a longer time-horizon and more complete hospital data.

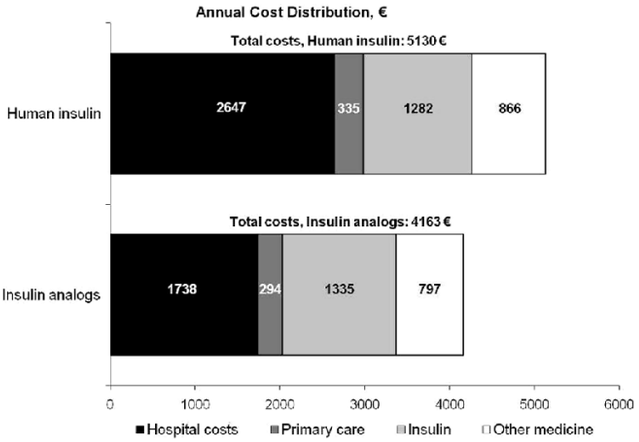

Figure. Follow-up annual healthcare costs 2005/6. Exchange rate: Average of 2006.

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Comparison of adherence and cost outcomes in patients with type 2 diabetes initiating rapid-acting insulin analogue with a prefilled pen versus vial/syringe

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Background and aims: Outcomes data on insulin pens compared to vial/syringe for rapid-acting insulin analog (RAIA) are limited in the literature. The aim of this research was to compare the adherence and cost outcomes of a newly available prefilled pen with insulin lispro vs. vial/syringe with insulin lispro or aspart in patients with type 2 diabetes (T2D). RAIA in premixed formulations were not included due to small sample size.
Materials and methods: A retrospective analysis was conducted using a US claims database. The study included patients who were ≥18 years old, new initiators of RAIA, with T2D, and ≥12-month continuous eligibility of medical and pharmacy benefits. After using a propensity score matching technique to match the 2 study cohorts, a difference-in-difference analysis was conducted by comparing the mean change in outcomes from 6 months prior (pre-index) to 6 months after (post-index) initiating the prefilled pen vs. vial/syringe. The Wilcoxon rank sum test was used to test for significance of the differences in outcomes across the 2 cohorts. Adherence was measured by dividing the number of days with RAIA by the 6-month post-index period (range: 0-100% with a higher percent indicating higher adherence). Cost outcomes (2009 US Dollars) included total costs, diabetes-related costs, and the subgroups of pharmacy, outpatient, emergency room (ER), and inpatient costs.
Results: Post-matched baseline patient characteristics including cost measures were similar between the prefilled pen (n=239) and vial/syringe (n=590) cohorts. Mean age and percentage of females were 59 vs. 60 years (p=0.22) and 47% vs. 48% (p=0.67), respectively. Adherence to the newly initiated RAIA in the post-index period was higher in the prefilled pen cohort than the vial/syringe cohort (55% vs. 45%; $p<0.001$). The increase in diabetes-related pharmacy costs after RAIA initiation was significantly greater in the prefilled pen cohort than the vial/syringe cohort (Table 1). A significant reduction in total diabetes-related costs was observed in the prefilled pen cohort when compared to the vial/syringe cohort (Table 1). There were no significant differences in changes to total costs, diabetes-related outpatient, ER, or inpatient costs between the 2 cohorts.
Conclusion: Our findings suggest that even with a greater increase in diabetes-related pharmacy costs, initiating RAIA with a prefilled pen was associated with greater adherence and lower total diabetes-related costs than vial/syringe. Further research is needed to elucidate the key drivers of this greater reduction in the total diabetes-related costs.

Table 1: Results of Diabetes-Related Pharmacy and Total Costs

Diabetes-related costs (mean, US Dollars)	Prefilled Pen			Vial/Syringe			
	Pre-Index	Post-Index	Difference from pre- to post-index	Pre-Index	Post-Index	Difference from pre- to post-index	p-value of difference-in-difference
Pharmacy costs	963	1,864	+901	880	1,486	+606	<0.001
Total costs	3,049	2,814	-235	2,914	2,975	+61	0.01

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German real-life data indicate lower costs for basal supported oral therapy (BOT) with insulin glargine compared to combination therapy with exenatide and oral antidiabetic drugs

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Background and aims: Clinical efficacy of the new antihyperglycaemic injectable exenatide (EXE) in combination with oral antidiabetic drugs (OAD) is reported to be comparable to basal supported oral therapy (BOT) with insulin glargine (GLA). This study investigated the costs of a combination therapy of exenatide (EXE) and OAD vs. a BOT with GLA in type-2-diabetics (T2D) in Germany based on real-life data.
Materials and methods: A historical cohort study was performed using a representative patient database (IMS® Disease Analyzer). T2D who initiated a BOT with GLA or a combination therapy with EXE and OAD between 1/2007 and 12/2008 and whose data were continuously documented at least 12 months before and 12 months after therapy initiation were included. The following variables were collected: age, gender, insurance status, region and specification of the practice, diabetes duration, HbA1c level and BMI. Resource utilization (RU) and costs (based on public prices) were determined for a time period of 12 months after initiation of therapy with GLA (BOT) and EXE (OAD), respectively. The diabetes-related direct treatment costs (DR-costs) were identified for both treatment regimens, containing GLA, EXE, OADs, glucose [i.v.]/glucagon, blood glucose test strips (BGT strips) and consumables such as lancets and needles. Additionally, direct costs for co-medication (antihypertensive, lipid lowering, antithrombotic and cardiovascular drugs) were assessed for GLA (BOT) and EXE (OAD). RU included the evaluation of the number of physician visits, referrals to specialists and hospital admissions. Applying regression analysis, adjusted RU and costs for GLA (BOT) vs. EXE (OAD) were calculated. The variables age, gender, specification of the practice, region, diabetes duration, HbA1c level and BMI were included into the model.
Results: 1,934 T2D were included, of which 1,484 received GLA (BOT) and 450 patients were treated with EXE (OAD). GLA-patients were older (70.2 years vs. 58.1 years; p<0.0001), had a longer diabetes duration (5.4 years vs. 3.9 years; p<0.0001), a higher HbA1c level (7.6% vs. 7.3%; p<0.0001) and a lower BMI (30.7kg/m^2 vs. 35.6 kg/m^2; p<0.0001) than EXE-patients at baseline. The unadjusted annual DR-costs were 1,068 € for GLA (BOT) and 1,740 € for EXE (OAD) (Δ = 672 €). The total adjusted annual DR-costs were lower in T2D on GLA (BOT) than on EXE (OAD); cost savings amounted to 640 € (p<0.0001) per year. This is mainly driven by the lower costs of GLA compared to EXE (Δ = 809 €; p<0.0001). Additionally, a cost advantage of GLA (BOT) vs. EXE (OAD) was found for the use of consumables (Δ = 37 € p<0.0001). In contrast, the adjusted cost of BGT strips were lower in EXE-patients than in GLA-patients (Δ = 203 €; p<0.0001). No significant differences were found for the expenses of OAD and co-medication, as well as for RU in both groups.
Conclusion: A randomized controlled trial showed similar efficacy of EXE and GLA, both in combination with OADs. This cost comparison yielded substantial cost savings in favour of the GLA treatment regimen. After adjustment the cost advantage of GLA remained stable. Therefore, the BOT treatment based on GLA compared to a combination therapy of EXE and OAD in T2D could lead to relevant cost savings in Germany.
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Resource utilization and diabetes-related treatment costs of type-1-diabetics treated with ICT based on insulin glargine, insulin detemir or NPH insulin in Germany

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Background and aims: The chronic course of the disease and the specific long-term complications of type 1 diabetes mellitus lead to substantial costs for the German health care system. Hence, the cost-effectiveness of different insulin formulations is gaining increasing importance. The aim of this study was to compare resource utilization and associated direct treatment costs of an intensified conventional therapy (ICT) with three different basal insulins in type-1-diabetics: human basal insulin (NPH), insulin glargine (GLA) and insulindetemir (DET).
Materials and methods: Type-1-diabetics who had started an ICT with NPH, GLA or DET between 7/2000 and 2/2008 were identifyed by using a representative German database (IMS® Disease Analyzer). Patients whose data were continuously documented at least 12 months before and 18 months after initiation of an ICT were included. Patients who had a prescription of premixed insulin or were switched to another basal insulin within the observational period were excluded. The following variables were collected: age, gender, diabetes duration, HbA1c-level, Body Mass Index (BMI), insurance status (private versus statutory), geographical region and specification of the practice. Resource utilization was determined for a time period of 12 months and included the evaluation of basal and bolus insulin, blood glucose test strips, number of physician visits (general practitioner and specialist) and hospital admissions. Diabetes-related direct treatment costs (insulin, test strips, lancets, pens, needles, glucose i.v., glucagon) were calculated based on public prices for patients receiving NPH, GLA and DET, respectively. Finally, the evaluated resources and costs were adjusted for the variables age, gender, diabetes duration, HbA1c-level, BMI, specification of the practice and region, applying a multivariate regression model.
Results: 2,740 type-1-diabetics were included, of which 1,218 received an ICT with NPH, 1,079 with GLA and 443 with DET, respectively. The unadjusted annual diabetes-related direct treatment costs were 1,308 € for NPH, 1,512 € for GLA and 1,729 € for DET. After adjusting for potential confounders ICT with GLA showed economic advantages compared to ICT with NPH (-234 €/year; p<0.0001) or DET (-425 €/year; p=0.2800). The consumption of basal insulin and test strips was lower in patients treated with GLA compared to NPH (-6.00 U/day; p=0.3514 und -0.31 units/day; p=0.8291) or DET (-3.23 U/day; p<0.0001 und -0.59 units/day; p=0.0235). The number of referrals to specialists was lower for patients with GLA than in DET treated patients (-0.51/year; p=0.0009) but higher than in NPH treated patients (0.16/year; p=0.9184).
Conclusion: After adjustment for potential confounders this analysis of German real-life data showed that the ICT with GLA is related to lower annual treatment costs than the ICT with NPH or DET. In view of the equal clinical efficacy as reported in several randomized clinical trials and the economic advantages in comparison to NPH or DET, GLA should be regarded as the favored therapeutic option for type-1-diabetics in Germany.
Supported by: sanofi-aventis Deutschland GmbH

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Acute day hospital for medical care of hyperglycaemic crises in the last years of life

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Background and aims: Hyperglycemic crises in oldest elder people have usually been treated in a hospitalization ward. The inhospital management is an expensive way of care and it may involve nosocomial complications. There are no studies comparing effectiveness and feasibility of Day Hospital treatment versus conventional hospitalization for patients older than 74 years. Aims: 1.To assess the effectiveness of treating acute complications of diabetes (ACD) in a day hospital (DH) versus conventional hospitalization (CH) in older elder patients. 2. To evaluate glycaemic control at 3-6 months in both

groups. 3. To compare short term re-admissions (3 months) due to diabetes and nosocomial morbidity between study groups.
Materials and methods: A prospective, non randomized, cohorts study with 6 months follow-up. We included all diabetic patients aged >74 years consecutively admitted in our hospital, with ketosis and/or acute hyperglycemia >300 mg/dl. We excluded patients presenting with HC03 < 14 mEq, pH < 7.20, blood glucose >600 mg/dl, hemodynamic instability, severe intercurrent illness (infectious, CV disease ....), or Activities of Daily Living > D (Katz Index). Patients were assigned to DH or CH according to time of admission to the emergency-room. If patients were admitted out of DH opening hours they were assigned to CH. AII patients included in the CH cohort could have been treated in DH if they had arrived during the DH-opening hours. Investigators did not participate in group assignment The cost calculation included structural cost and all visits (emergency ward, day hospital and outpatients consultations).
Results: During the four years study period, 101 patients met the inclusion criteria. 64 were attended in DH and 37 in CH. The average age was 80.5 ± 4.7 years (range 75-95), and there were 62.7% females, without differences between the groups. Cause of admission was acute hyperglycemia in 63.4% and Ketosis in 36.6%. Infection (37.5%) was the most common cause of ACD, followed by new onset diabetes (11.5%) and corticosteroid therapy (12.5%). During the first 15 days after dischange we observed 4 severe hypoglycemic events without differences between groups. The average cost per patient and year was 584,9 euros for the DH cohort and 1.796,0 euros for the CH group.
Conclusion: Our results prove the effectiveness and safety of ACD management in Day Hospital with a net economic saving of 1.211,1 euros/case with no differences in short term glycaemic control and hypoglycemic events, and with less frequent readmissions and pressure ulcers.

	DAY HOSPITAL	Hospitalization	significance
Charlson index	3.22 ± 2.0	3.22 ± 1.7	p>0.05
Katz index (A or B%)	73	72.2	p>0.05
Blood Glucose (mg/dl)	420 ±74.8	449 ± 97.8	p>0.05
PH	7.39 ± 0.05	7.39 ± 0.06	p>0.05
HbA1C (baseline)	10.9 ± 1.92	10.7 ± 2.1	p>0.05
HbA1C (3 months)	7.6 ± 1.1	7.9 ± 1.0	p>0.05
HbA1C (6 months)	7.3 ± 0.7	7.6 ± 0.6	p>0.05
hypoglycemic episodes (3 months)	1.1 ± 1.8	0.4 ± 0.51	p>0.05
pressure Ulcer (% patients)	0	16	p>0.05
Readmissions (3 months) (% patients)	6.3	20	p>0.05
Follow up visits	4.9 ± 2.2	2.6 ± 1.9	p>0.05

Supported by: Spanish Health Ministry Fundació Agrupaciómutua: ambit de

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Cost-effectiveness analysis of medical intervention in patients with early detection of diabetic retinopathy in a tertiary care hospital in Bangladesh
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Background and aims: The economic burden resulting from diabetic retinopathy (DR) consumes a major portion of resources allocated for health-care services. Cost-effectiveness of various interventions on DR and its complications has relatively been well explored in developed countries, but these are almost absent in developing countries. The present study was undertaken to assess the cost-effectiveness of medical intervention in patients with DR.
Materials and methods: Two hundred patients with DR, with at least 1 year of follow-up, were purposively selected from the Out-Patient Department of BIRDEM (tertiary diabetes care hospital), Bangladesh. Of them 100 were late in detection of DR (LDR) & 100 were detected early (EDR). The degree & extent of complications like cardiopathy, peripheral neuropathy, nephropathy & vasculopathy, treatment outcome, clinical effectiveness of interventions and direct, indirect & incremental cost of complications were calculated. Comparison was made between the groups. Cost included drugs, hospitalizations, diagnostics & visits.
Results: A total of 200 patients were considered for an average of 365 days, amounting to 656 person-years of observation in total. In LDR group, 42.4% had mild nonproliferative DR (NPDR), 31.4% had moderate, 15.1% had severe NPDR & 11.1% had proliferative DR (PDR). In EDR group, 58.4% had mild and 41.6% had moderate NPDR. The mean±SD fasting serum glucose of the groups (LDR & EDR respectively) was 9.36±0.40 & 4.78±0.38 mmol/l, total cholesterol was 206.50±42.60 & 104.20±35.50 mg/dl, HbA_{1c} was 9.80±0.50% & 5.70±0.38%, TG was 163.76±99.46 & 155.67±94.84 mg/dl, SBP was 172.5 ± 20.9 & 109.5±11.9 mmHg and DBP was 97.7±10.0 & 70.7±9.3 mmHg. About 17% patients in LDR & 34% in EDR were free of diabetic complications other than DR. In LDR & EDR, 18% & 46% had one complication, 27% & 8% had two and 30% & 4% had more than two complications respectively. The most frequent complication was cardiopathy, which affected 31% patients in LDR & 25% in EDR, followed by peripheral neuropathy 19% & 16%, nephropathy 15% & 11%, and vasculopathy 8 % & 4% respectively. The average annual cost of care was US$ 27954 (direct US$ 16983 & indirect US$ 10971), with an average US$ 140 per patient. Among the average annual cost LDR consumed US$ 19737 (US$ 197 per patient) & EDR US$ 8217 (US$ 82 per patient). US$ 13473 (48%) of costs was attributable to drugs for both groups of which US$ 10817 (80%) was for LDR & US$ 2656 (20%) for EDR, US$ 8739 (31%) to hospitalizations of which US$ 5211 (60%) for LDR & 3528 (40%) for EDR. In case of diagnostics & visits the corresponding values were US$ 2136 (60%) & 1419 (40%) and US$ 1673 (76%) & 514 (24%) for LDR & EDR respectively. The annual medical costs increased with the increased number of complications from US$ 1322 to 2298 & to 3991 in LDR with one, two & more than two complications (other than DR) which is increasing at a rapid rate and US$ 917 to 1556 & to 2372 in EDR respectively, increasing at a diminishing marginal rate. The regression equation showed that medical cost is significantly related to complications tested in both univariate ($P<0.0001$) & multiple linear regression analyses ($R^2=0.53$; $F=82.3$, $P<0.0001$).
Conclusion: Proper management with regular screening substantially reduces the expenditure related to care of patients with diabetic retinopathy & related complications even in a developing country. Strategies aimed at preventing DR & early detection of the onset of retinopathy will reduce medical costs in a substantial way.

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Assessment of medication adherence among patients with type 2 diabetes
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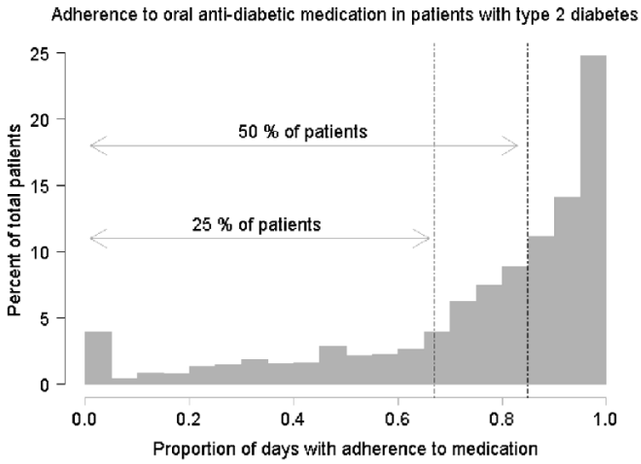
Background and aims: Poor adherence to prescribed medication may markedly limit physicians' ability to achieve and maintain adequate glycaemic and cardiovascular risk control in patients with diabetes. However, much remains unknown about patterns of adherence to treatment in patients with complex treatment strategies. We sought to assess and summarize patterns of medication adherence to oral anti-diabetic medication in patients with type 2 diabetes (T2DM), attending a specialised diabetes hospital in Copenhagen.
Materials and methods: Registrations of drug prescriptions issued by physicians at the outpatient clinic for type 2 diabetic patients followed for at least two years between 2002 and 2007 were linked to registrations of filled prescriptions at Danish pharmacies at an individual level. Medication episodes were defined based on prescriptions issued at the hospital. Within each episode the number of days with and without adherence was calculated based on pharmacy data. A binomial regression model was used to assess the association between the degree of adherence and sex, age and duration of diabetes.
Results: 1,634 patients with T2DM (60.5% men, mean age at entry: 59.4 years (men), 60.3 years (women), mean duration of diabetes at entry: 9.7 years (men), 10.5 years (women), with an average clinic attendance duration of 4.3 years contributed with over 7,000 person-years of time. The median degree of adherence was 0.85. An adherence ratio of 0.73 and above was achieved by 75% of the patients. More than 15% of the patients were adherent to treatment less than half the time. We found a higher degree of adherence by increasing age. Women at age 60 with a duration of diabetes of 10 years had a 2.6 %-points higher degree of adherence than women at age 50. There was no statistically significant difference between men and women or according to duration of diabetes.
Conclusion: The degree of adherence to oral anti-diabetic medication ranged from not picking up any prescription to full adherence. Only age was positively associated with the degree of adherence. Further explanatory factors such as socio-economic variables and analysis of adherence patterns to statins, antithrombotic and anti-hypertensive medication deserve consideration. Our findings indicate that a considerable improvement in glycaemic control can be achieved by improving adherence to medication.



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Foetal exposure to maternal diabetes is associated with insulin secretory defect in females at adult age

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Background and aims: In humans, excess maternal transmission of type 2 diabetes supports the hypothesis that the intrauterine environment contributes to increased risk of type 2 diabetes in offspring. We have shown that fetal exposure to maternal diabetes is associated with an insulin secretion defect in response to glucose at adult age. The aim of the present study was to investigate whether fetal exposure to maternal diabetes is associated with a global (endocrine and exocrine) pancreatic dysfunction in non diabetic adult offspring.
Materials and methods: We investigated offspring of type 1 diabetic patient to circumvent the confounding effect of genetic factors related to type 2 diabetes. 29 adult offspring exposed to maternal type 1 diabetes during pregnancy (exposed group) were compared with 29 offspring of type 1 diabetic fathers (control group). Early insulin secretion in response to oral glucose defined as the ratio of Δ insulin $_{0\text{-}30}$ to Δ glucose $_{0\text{-}30}$ was measured during OGTT. Insulin and glucagon secretion were assessed during graded glucose infusion from 4 to 16 mg/kg/min followed by a 5-g arginine bolus. Insulin action was measured using a euglycemic hyperinsulinaemic clamp and percent body fat mass by DEXA. Exocrine pancreatic function was evaluated by quantitative stools analyses.
Results: Mean age, sex ratio, mean percent body fat and mean insulin sensitivity were similar in the exposed and control groups: 25.9 ± 6.2 (SD) vs 26.2 ± 6.1 years, 55 (F/M) vs 52 %, 26.3 ± 8.7 vs 24.5 ± 7.9% fat mass and 11.5 ± 2.9 vs 11.7 ± 2.5 mg/kg of body fat free mass/min respectively. Impaired glucose tolerance was diagnosed in two offspring in each group. Early insulin secretion was lower in the exposed group than in the control group: 7.8 (median) (5.5-10.6 Q1-Q3) vs 11.3 (6.4-17.1) µUI/mmol (p = 0.06). In response to IV glucose and arginine there was no difference between the 2 groups with respect to insulin secretion and glucagon concentrations. However, women exposed in utero to maternal diabetes had a significantly decrease in insulin secretion rate compared to those of the control group: 12.5 ± 4.5 vs 15.2 ± 5.2 pmol/kg/min (p=0.03). Fecal fat output was similar in the 2 groups but fecal chymotrypsin activity was significantly lower in the exposed group: 14.5 ± 7.2 vs 22.8 ± 13 U/g (p = 0.016).
Conclusion: Fetal exposure to maternal type 1 diabetes seems to be associated with a global pancreatic dysfunction at adult age. Insulin secretory defect in response to IV glucose is only observed in women suggesting sex-dependent epigenetic mechanisms.
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Glycaemic control, pre-eclampsia and gestational hypertension in pregnant women with type 1 diabetes

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Background and aims: An association between glycaemic control and preeclampsia has been reported but the results are conflicting and the rela-

tive importance of early and late control for hypertensive complications of pregnancy remains unclear. The aim of this study was to assess the relationship between glycaemic control, preeclampsia and gestational hypertension in women with type 1 diabetes.
Materials and methods: HbA1c measurements were available from women taking part in the Diabetes and Preeclampsia Intervention Trial (a multicentre randomized controlled trial investigating the effect of antioxidants on the incidence of preeclampsia) at up to 6 months pre-pregnancy, at the first antenatal visit (booking), and at 26 and 34 weeks gestation. Results were categorized as poor (>8%), moderate (7-8%) and good (<7%) glycaemic control. Preeclampsia and gestational hypertension were defined using the International Society for the Study of Hypertension in Pregnancy guidelines. Logistic regression was used to estimate the odds on preeclampsia and gestational hypertension in women with poor and moderate control relative to women with good control both before and after adjustment for potentially confounding variables.
Results: Preeclampsia and gestational hypertension developed in 17% and 11% of pregnancies, respectively. Poor/moderate glycaemic control both before and during pregnancy were associated with significantly increased risk of preeclampsia compared with good glycaemic control. After adjustment for confounding variables the association between HbA1c and preeclampsia remained significant throughout pregnancy with highest odds ratios observed in the last trimester (see table). Glycaemic control during pregnancy was not significantly associated with gestational hypertension either before or after adjustment for confounders.

Glycaemic control before and during pregnancy and risk of preeclampsia and gestational hypertension

Time-point	n	Glycaemic control (HbA1c)	Preeclampsia*	Gestational hypertension*
Pre-pregnancy	542	<7%	1.00	1.00
		7-8% vs <7%	1.70 [0.79-3.64]	0.62 [0.30-1.29]
		>8% vs <7%	2.53 [1.19-5.36]	0.72 [0.36-1.43]
First antenatal visit	721	<7%	1.00	1.00
		7-8% vs <7%	2.29 [1.22-4.31]	1.00 [0.54-1.86]
		>8% vs <7%	3.46 [1.83-6.55]	0.91 [0.49-1.72]
26 weeks gestation	592	<7%	1.00	1.00
		7-8% vs <7%	2.01 [1.17-3.47]	0.80 [0.41-1.54]
		>8% vs <7%	2.15 [0.84-5.50]	0.79 [0.26-2.46]
34 weeks gestation	519	<7%	1.00	1.00
		7-8% vs <7%	2.32 [1.14-4.72]	1.09 [0.53-2.24]
		>8% vs <7%	5.87 [1.64-21.1]	0.79 [0.16-3.80]

*Adjusted Odds Ratio [95% Confidence Interval]. Adjusted for: treatment group, centre group, BMI, diabetes duration, parity, smoking, age, plasma ascorbate and serum α-tocopherol at randomisation, microalbuminuria before pregnancy.

Conclusion: Poor glycaemic control is associated with an increased risk of preeclampsia but not gestational hypertension. While glycaemic control is important before and throughout pregnancy, HbA1c during the last trimester of pregnancy is the strongest predictor of preeclampsia.
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1070

In women with type 1 diabetes mellitus, maternal insulin requirements are potent predictors of excess fetal growth
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Background and aims: Excessive fetal growth remains as an important issue in diabetic pregnancies, glycemic control being one among other predictor variables.
Materials and methods: To perform a comprehensive analysis of potential predictors of excessive fetal growth Singleton pregnancies of women with T1DM delivering in the center between January 1981 and December 2006 and receiving the same treatment schedule (multiple injections/insulin pump; regular/lispro insulin) since before pregnancy until delivery. Outcome variables: macrosomia (birthweight >=4000g), large for gestational age newborn (LGA, >P90 of national growth charts). Potential predictors: maternal anthropometric variables, smoking habit, obstetric history (prior pregnancies, macrosomia, poor obstetric history), maternal hypertension, fetal sex, delivery-related variables (gestational age, calendar year and number of pregnancies in that year) and diabetes-related variables (duration, treatment, microangiopathic complications, prepregnancy care and mean blood glucose, insulin requirements (IR, IU/kg/d) and glycated haemoglobin in 1st, 2nd and 3rd trimesters of pregnancy). Statistics: Logistic regression analyses were performed (backward method) with macrosomia and LGA as outcome variables and all aforementioned variables as potential predictors.
Results: 315 consecutive pregnancies fulfilling entry criteria were included. The rate of macrosomia was 13.3% and that of LGA 38.6%. In the prediction model of LGA, 9 predictors were identified including 3 diabetes-related variables (1st trimester IR (OR 72.4, CI 2.4, 2208), 3rd trimester IR (0.04, CI 0.001, 1.043) and 3nd trimester HbA1c (2.871, CI 1.438, 5.732)). The prediction model of macrosomia was consistent with that of LGA.
Conclusion: In women with T1DM, maternal IR are potent predictors of excess fetal growth. The association with 3rd trimester IR can be a consequence of fetal siphoning in overgrown babies whereas the association with 1st trimester IR suggests a causal role of maternal insulin.
Supported by: CIBER-BBN

1071

Pregnancy outcomes in women with type 1 and type 2 diabetes in a polish population
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Introduction and aim: The number of pregnant women with type 2 (T2DM) is increasing worldwide. However, the majority of scientific reports on pregestational diabetes is associated with type 1 diabetes (T1DM). The knowledge on pregnancies of T2DM women is still incomplete. The purpose of this observational study was to assess glycemic control and pregnancy outcomes in women with pregestational T2DM and to compare them with T1DM.
Methods: Medical records of 415 consecutive singleton pregnancies in women with pregestational diabetes from 1999 to 2009 were analysed at the Department of Metabolic Diseases, Krakow, Poland. All women were Caucasians. Among them, there were 70 women with T2DM and 345 with T1DM. We compared HbA1c levels as well as selected maternal and foetal outcomes in both groups.
Results: Compared to T1DM, women with T2DM were significantly older (mean 33.1 years±5.2 vs. 27.8±5.0, respectively), heavier before pregnancy (mean weight 80.7 kg±17.8 vs. 64.5±10.1) and had shorter duration of diabetes (mean 3.3 years±3.2 vs. 11.5±7.3); (p=0.00001 for all comparisons). T2DM women less weight gain during pregnancy than T1DM (mean 10.4 years±7.6 vs. 13.8±6.5; p=0.0001), but final body weight before delivery were higher in T2DM group (mean 90.6 years±16.9 vs. 78.2±11.3; p=0.0000). The gestational age of the first visit was higher in T2DM women (mean 11.4 weeks±7.1 vs. 8.6±4.5, respectively; p=0.00001). Nevertheless, they had better glycemic control in the 1st trimester as measured by HbA1c (6.1% ±1.1 vs. 6.9±1.6; p=0.0008). We observed a decrease of HbA1c level in both groups in the 2nd (5.7 % ±0.9 vs. 5.9±0.8) and 3rd trimester (5.6 % ±0.6 vs. 5.8±0.9), the differences in HbA1c were no longer significant. The rate of perinatal mortality (2.9% vs. 3.5%) and major congenital malformations (7.1% vs. 6.1%) as well as the proportion of pregnancies ended by caesarean sections (56.3% vs. 63.9%) were similar in both groups. The birth weight was slightly smaller in T2DM than in T1DM (mean weight 3194.6 g±880 vs. 3419.2±681.3; p=0.04). The rate of babies born before 37 week of pregnancy was comparable in both groups, respectively 12.9% vs.17.1%. We also observed a similar rate of stillbirths in both groups (8.6% vs. 7.0%; p=0.4).
Conclusion: In this large observational study we found similar pregnancy outcomes in women with T1DM and T2DM in a Polish population, despite better glycemic control at the beginning of pregnancy in T2DM.
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1072

Pre-pregnancy body mass index and the risk of adverse pregnancy outcome in two thousand type 2 diabetes mellitus Bangladeshi women

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Background and aims: Obesity before pregnancy is associated with an increased risk of late fetal death, early neonatal death, preeclampsia & hypertensive disorders, preterm delivery, shoulder dystocia & macrosomia. The mother's being leaner than average on the other hand, is associated with an increased risk of delivering an infant of small for gestational age (SGA). The study was undertaken to assess the effect of the pre-pregnancy body mass index (BMI) on maternal & fetal outcome of 2000 type 2 diabetes singleton pregnant women who attended the obstetrics-diabetology Out-Patient clinic of a tertiary care hospital in Bangladesh.
Materials and methods: The women were categorized according to their BMI (kg/m^2): lean < 20.0, normal from 20.0 to 24.9 and obese > 30.0 kg/m^2. Information regarding maternal age, parity, complications during pregnancy or delivery and perinatal outcomes were obtained from hospital records. Late fetal death was defined as still birth occurring at 28 or more completed weeks of gestation and early neonatal death as death occurring during the first week after birth, preterm delivery was less than 37 completed weeks of gestation. SGA infants were defined as the birth weight more than 2SD below the mean birth weight for gestational age. Gestational age was estimated as based on ultrasound examination performed routinely at less than 12 weeks of gestation. The estimates were adjusted for maternal age, parity, smoking, education, and weight gain during pregnancy. The effect of pre-pregnancy BMI was analyzed by comparing the frequencies of various outcomes in three BMI groups by both univariate and multivariate logistic regression analysis. The results were expressed as odds ratio (ORs) and the corresponding 95% confidence intervals (CIs) & p values.
Results: The mean±SD age of the study subjects were 34±5 years, the median (range) duration of diabetes was 4 (3-5) years. The risk of late fetal death was consistently increasing with BMI (ORs were 1.2 (0.9-1.7), 1.6 (1.1-2.3) & 2.6 (1.7-3.8) for lean, normal & obese respectively). The risk of early neonatal death was also higher among women with higher BMI (ORs was 1.6 (1.1-2.3) for obese) (p<0.001). The rate of preeclampsia increased with increasing BMI (the values were 1.8%, 2.5% & 7.0% for lean, normal & obese respectively). Hypertensive disorders was also more common among obese (4.6%) compared with lean (1.3%) and normal (2.6%) (ORs 3.8 (2.5-5.6), 1.6 (1.1-2.2) & 2.5(1.7-3.5) respectively). The risk of preterm delivery was significantly increased for obese group (4.2%), as compare to lean (2.2%) & normal weight (2.4%) (ORs 1.6 (1.3-2.1), 1 (0.8-1.6) & 1.2 (0.9-1.6) respectively) (p<0.001). The risk of SGA was significantly more in lean (2.7%) compared to normal weight (1.5%) & obese group (1.9%) (ORs 2.2 (1.7-2.8), 1.2 (0.9-1.5) & 1.0 (0.6-1.3) respectively). The risk of shoulder dystocia & macrosomic baby was higher in obese group.
Conclusion: Pre-pregnancy obesity increases the risk of late fetal death and perinatal mortality. As obesity prevents small for gestation age infant in Type 2 Diabetes subjects, the Type 2 DM lean women were advised to take adequate diet to meet the basic requirements of pregnancy. On the contrary obese women should reduce the body weight before pregnancy.

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What is determined by impaired cardiac function in pregnancy with gestational, types 1 and 2 diabetes mellitus: maternal or neonatal prognosis7

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Background and aims: Cardiac autonomic neuropathy is a common dysfunction in manifest diabetes mellitus (DM) and is proportional to the duration of diabetes and/or a poor glycaemic control. Heart rate variability (HRV) reflects autonomic heart function. The aim of the present study was to investigate whether in pregnant women with prior gestational DM (GDM), insulin-dependent DM (IDDM) and insulin-independent DM (IIDM) alterations of cardiac autonomic function can be observed at the 37-39 weeks of gestation in relation to maternal or neonatal prognosis.
Methods: Fifty four women (10 with GDM - group 1, 17 with IIDM - group 2, 13 with IDDM until 5 years duration - group 3, 7 with IDDM from six to fifteen years duration - group 4 and 7 with IDDM more, than sixteen years duration - group 5) underwent 24-h Holter monitoring at the 37-39 weeks of gestation and 3-6 days postpartum. Heart rate variability (HRV) measures derived from 24-hour electrocardiography monitoring, calculated in the time (standard deviation of all normal RR intervals (SDNN), standard deviation of 5-minute RR intervals (SDANN), root-mean-square of difference of successive RR intervals (rMSSD), and percentage of adjacent RR intervals >50 ms different (pNN50) and frequency domain (total power - TP, power within low-frequency band - LF, and power within high-frequency band - HF).
Results: In group 3 HRV was higher: SDNN was 122,4±24,4ms vs 86,0±34,3ms in GDM; 110,7±60,5ms in group 2; 104,5±40,6ms in group 4 and 50,2±6,7ms (p<0,05) in group 5 respectively. HRV patients of groups 2 and 4 were characterized by wide dispersion of HRV, which reflected from other diseases (hypertension, preeclampsia etc). In type 1 diabetes mellitus HRV progressively declines with duration of illness. Extremely low activity of sympatic nervous system (SDANN less than 68ms) in the 5 group was connected with lower Apgar score, but in common with higher parasympathic activity (rMSSD more than 45 ms) - with poor neonatal and maternal prognosis. After delivery there were no observed restoration HRV to normal in women with diabetes type 1.
Conclusion: The loss of the variability of the cardiac rhythm confirms that in women with IDDM has a chaos in cardiac pacing, which reflects autonomic neuropathy and other complications. In type IDDM neonatal prognosis correlates with maternal activity of sympatric nervous system.
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1074

Hypertension and end stage renal disease in women with a past history of gestational hypertension

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Background and aims: Insulin resistance is thought to be a converging point in the pathophysiology of GH and it is postulated that women with this disorder have a much higher chance of developing hypertension and end stage renal disease (ESRD) at a later period after delivery. Prospective studies on this issue, however, are still limited. The aim of the present study was to investigate the long-term effect of GH on the postpartum development of hypertension and ESRD.
Materials and methods: The study design had both a cross- sectional and retrospective component. A total of 140 women [age in years 32.4±8.1(yrs) and BMI 25.1±4.1(kg/m^2, m±SD)] with a previous history of GH in any pregnancy were included. Clinical and anthropometric parameters were measured by standard techniques, lipids were measured by enzymetic colorimetric method, urinary total protein by pyrogallol red method, urinary protein by strip method and serum urinary creatinine were measured by alkaline picrate method. Systolic blood pressure 130 mmHg or diastolic blood pressure 90mmHg were taken as cut-off values for hypertension and urinary protein >35mg/dl was the marker for ESRD.
Results: Out of the 140 subjects 49 (35%) developed hypertension and 46 (32.9%) developed ESRD over duration of 5 to 12 yrs. 45(32%) of the subjects had both the complications. The hypertensive subjects had higher age (years, m±SD, 35.8±9.7 vs 30.5±6.4, p=0.001), BMI (kg/m^2 , 26.09±4.3 vs 24.6±3.9; p=0.045), uric acid (mg/dl, 7.38±1.1 vs 4.6±1.6; p=<0.001) and total protein (mg/dl, 47.7±13.5 vs 15.5±5.4; p=<0.001). On logistic regression analysis, hypertension showed a strong positive association with uric acid and total protein when the effects of age and BMI were adjusted. On the other hand ESRD showed strong positive association with uric acid when the effects of age, BMI, fasting blood sugar and Triglyceride were adjusted.
Conclusion: Women with history of GH has a high probability of developing hypertension and ESRD in postpartum life, and both the conditions seem to have association with uric acid as a risk factor.
Supported by: BADAS

1075

ATLANTIC DIP: persistent postpartum glucose intolerance in women with previous gestational diabetes along the Irish Atlantic seaboard

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Background and aims: Gestational diabetes / Impaired Glucose Tolerance (GDM/IGT) is associated with adverse fetal and maternal outcomes. It also identifies women at risk of developing IGT and Type 2 diabetes (T2DM) later in life and in the postpartum period. Up to date prospective figures are not available for persistent glucose intolerance postpartum in the Irish population.
Materials and methods: We compared 357 women with abnormal (GDM/IGT) and 137 women with normal (NGT) glucose tolerance in pregnancy identified by 75g oral glucose tolerance test (OGTT) at 24-28 weeks gestation. This was repeated post partum to reassess glucose tolerance. Logistic regression analysis was used to identify maternal factors that increased the risk of persistent glucose intolerance.
Results: 494 women were tested. OGTT results were classified as NGT (FPG<5.6mmol/l; 2h<7.8mmol/l) or abnormal (IFG; 5.6-6.9, IGT; 2h 7.8-11.0, IFG+IGT; T2DM FPG≥7 ± 2h≥11.1). 2 of 137 (1.4%) women with NGT in pregnancy had abnormal glucose tolerance postpartum. 54 of 357 (15.1%) women with abnormal glucose tolerance (GDM/IGT) in pregnancy remained glucose intolerant post partum. Risk factors for persistent glucose intolerance were family history of T2DM (OR 2.92, 95% CI 1.15-7.41, P=0.02), insulin use in pregnancy (OR 3.65, 95% CI 1.41-9.45,P=0.007). Fasting plasma glucose in pregnancy of 5.6-6.9mmol/l (OR 3.73, 95% CI 1.25-11.09, P=0.01) and ≥7(OR 16.89, 95% CI 3.31-86.02, P<0.001) were strong predictors of postpartum dysglycaemia. Age, BMI, ethnicity did not predict persistent dysglycaemia.
Conclusion: Along the Irish Atlantic seaboard the prevalence of persistent glucose intolerance in women with GDM/IGT in pregnancy is 15.1% compared to 1.4% in control women. This high prevalence suggests a robust follow up programme is necessary for early identification and intervention.

Pregnancy and postpartum glucose status

Pregnancy glucose status	Postpartum glucose status				
	Normal	**IFG**	**IGT**	**IFG+IGT**	**T2DM**
Normal (n=137)	135 (98.5%)	1 (0.7%)	0	1 (0.7%)	0
IGT (n=263)	237 (90.1%)	14 (5.3%)	5 (1.9%)	4 (1.5%)	3 (1.1%)
GDM (n=94)	66 (70.2%)	7 (7.45%)	5 (5.3%)	10 (10.6%)	6 (6.4%)
Total (n=494)	438	22	10	15	9

Supported by: HRB

PS 103 Pregnancy - outcomes II

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Perinatal outcome in women with gestational diabetes mellitus in relation with fetal sex

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Background and aims: Male sex is a well-known risk factor for unfavorable perinatal outcome that has only occasionally been assessed in diabetic pregnancy. The aim of this study was to evaluate perinatal outcome in women with gestational diabetes mellitus (GDM) according to fetal sex.
Materials and methods: Database review including all singleton pregnancies of women with GDM progressing to >= 22 weeks, delivering in the center between 01/01/1981 and 31/12/2006. Evaluated maternal characteristics: anthropometrics, obstetric history, diagnosis characteristics (gestational age, blood glucose values), HbA1c (after diagnosis and in the third trimester). Outcome variables: preterm birth, abnormal Apgar, large and small for gestational age newborns, obstetric trauma, major and minor malformations, polycythemia, neonatal hypoglycemia, hypocalcemia, jaundice, respiratory distress and fetal loss (intrauterine, neonatal, perinatal). Statistics: Chi-square, Student T and Mann-Whitney U tests. Significance was set at a two-sided p <0.05.
Results: A total of 2216 pregnancies were included. Maternal characteristics did not differ between groups except for a higher maternal weight in male newborns (59 vs. 58) and a higher rate of prior pregnancy in female newborns (65.5 vs. 61.1%). Higher figures were observed in 13 out of 15 perinatal outcome variables in male newborns but statistical significance was not reached in any of them.
Conclusion: In this group of women with GDM, perinatal outcome is not significantly worse in male newborns.
Supported by: CIBER BBN

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Prevalence and outcome of gestational diabetes in Turkmenistan

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Background and aims: Very few data are available about the health care system in Turkmenistan a central Asian country with huge gas resources and rapidly increasing wealth and rates of obesity. As for other central Asian countries the prevalence of gestational diabetes (GDM) is unknown. The aim of this investigation was to prospectively determine the prevalence of GDM in Turkmenistan and the frequency of complications in newborns from GDM mothers.
Materials and methods: From March 2008 until September 2009 all pregnant women presenting to the perinatal center at the Ene Maehri Merkezi Hospital (University of Ashgabat) obtained a glucose screening (after 26 weeks of pregnancy; 50 g glucose orally). If the 60-min glucose concentration was ≥ 7.8 mmol/l an oral glucose tolerance test (75 gr) was performed. GDM was diagnosed if one or more glucose values were abnormal (≥5.0, ≥10.0, ≥8.0 mmol/l at 0-, 60-, 120-min, respectively). Birth weight, APGAR and 30 min glucose concentration was determined in all newborns.
Results: 25.4% of 1271 screened patients had a pathological screening test. Of those, 28.5% had GDM (overall prevalence 7.3%). Screening glucose (60-min) correlated with age (r=0.13; p>0.001), BMI (r=0.12, p<0.001), gravidity (r=0.12, p<0.001) and blood pressure (r=0.06, p=0.03). GDM patients were older (30.2±5.3 years vs. 27.1±4.9 years; p<0.001) and more obese (BMI 27.7±4.9 vs. 26.5±4.5 kg/m^2; p=0.03) than controls. GDM patients obtained more frequently scheduled caesarean sections (12.0% vs. 8.9%, ns) and less frequently emergency caesarean sections (8.8% vs. 13.3%, ns). In newborns delivered after ≥37 weeks gestational age (controls vs. GDM) birth weight (3500±462 vs. 3605±409 g, p=0.06) and APGAR (8.4±1.5 vs. 8.3±1.3, ns) did not differ between both groups but GDM children had more often hypoglycaemia (13.9% vs. 27.3%, p<0.05).

Conclusion: In Turkmenistan GDM is characterized by the same risk factors as in European countries. Because of the rapidly increasing wealth and increasing prevalence of obesity the prevalence of GDM will probably further increase. Newborns from GDM patients tended to be heavier and had high rates of hypoglycemia. This study shows that medical prevention programs can also be successfully implemented in Turkmenistan.

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Analysis of pregnancy outcomes in immigrate women with gestational diabetes

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Background and aims: Immigration is growing in all European countries, the populations diversity poses specific problems related to health care service and socio-demographic factors. Recent studies show adverse outcomes of pregnancy among immigrant women from countries with high diabetes rates. So we found of interest to analyse the outcomes in immigrate pregnant women affected by GDM compared to Italian ones.
Materials and methods: We compared maternal and fetal outcomes in 94 immigrant (ImPW) and1246 Italian women (IPW) with GDM followed up at our center. Maternal characteristics considered were age, pre-pregnancy BMI, HbA1c, frequency of insulin treatment, timing and mode of delivery, and hypertensive disorders; and, for fetal outcome, infants large(LGA) or small(SGA) for gestational age and fetal complications.
Results: Pre-pregnancy BMI (26.9 ± 4.6 Kg/m^2 vs 24.8 ± 5.3 Kg/m^2, p<0.0001) and HbA1c (at diagnosis and at 3rd trimester 5.6 ± 0.6% vs 5.2 ± 0.6%, p<0.0001) were higher in ImPW than in IPW, and more of them were on insulin (26.6% vs 17%, p=0.024).Gestational age at screening was not different: 23.6±5.8g.w. in ImPW vs 24.6±5.4 g.w. in IPW No differences in time and mode of delivery(cesarean section 46.1% in immigrate women vs 42.9% in Italian ones) and hypertensive disorders (6.8% vs 8.8%) emerged between the 2 groups. A higher rate of LGA babies (30.3% vs 19.8%, p=0.03) were born to immigrant women than to Italians, but fetal morbidity was not different (4.2% vs 6.5%).In a regression logistic analysis LGA newborns were related to maternal age (p=0.002) and HbA1c at 3rd trimester.
Conclusion: Our ImPW show higher glucose levels during pregnancy, but their outcome could be considered satisfied and comparable with IWP, probably because these women were subjected to GDM screening, diagnosis and treatment at the recommended gestational age . So immigrant GDM women have favorable outcomes if given access to health care, language and cultural barriers are removed.

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HbA_{1c} above normal range in late pregnancy is associated with risk of infants' large-for-gestational age in women with gestational diabetes mellitus

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Background and aims: HbA_{1c} is widely used as a measure of metabolic control during pregnancy and documented to be associated with diabetes related pregnancy complications in type 1 diabetes. In addition HbA_{1c} can be measured independent of the patients' compliance to glucose monitoring and is therefore of special value in women where the compliance to treatment is sub optimal. The value of using HbA_{1c} as a treatment goal and risk marker of complications in the newborns of women with GDM is to our knowledge not previously described in the literature. The aims of the present study were 1) to determine the prevalence of pregnant women with gestational diabetes mellitus (GDM) not obtaining HbA_{1c} within normal range before delivery and 2) examine whether elevated HbA_{1c} values are associated with increased risk of large-for-gestational age (LGA) infants.
Materials and methods: The study population was 148 GDM women delivering during 2007 at Rigshospitalet. Inclusion criteria; GDM diagnosed <34 weeks, singleton pregnancies and at least two HbA_{1c} test with more than 3 weeks interval. Data was extracted from medical records. The study population was divided in those obtaining HbA_{1c} less than or equal to 5.6% or not. The primary outcome was LGA infants. Secondary outcomes were preeclampsia, preterm delivery, birth complications, neonatal hypoglycaemia, respiratory distress syndrome and jaundice.
Results: Fifty-one (35%) women did not obtain HbA_{1c} less than or equal to 5.6% before delivery. The median HbA_{1c} before delivery was 5.9% (range 5.7-6.6) vs. 5.3% (4.5-5.6) in the two groups. At baseline, BMI and HbA_{1c} were higher in the women not obtaining the goal compared to the remaining women (30.9 (SD6) vs. 27.8 (SD7); 5.9% (5.7-7.8) vs. 5.1% (4.3-6.3)). Women with elevated HbA_{1c} before delivery were characterised by a higher risk of LGA infants and neonatal hypoglycaemia (adjusted OR 3.1 (95% CI 1.3-7.6), and 6.2 (95% CI 1.3-29.0)). Other secondary outcomes were similar in the two groups.
Conclusion: Women with GDM not obtaining HbA_{1c} within normal range before delivery had a three-fold increased risk of LGA infants and six-fold increased risk of neonatal hypoglycaemia.

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The prevalence of large for gestational age offspring and pregnancy complications before and after implementation of a new insulin treatment guideline in women with gestational diabetes

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Background and aims: The best strategy for insulin treatment of women with gestational diabetes (GDM) under routine conditions is not well described. In 2008 we decided to change from biphasic human insulin (Mixtard) to biphasic insulin aspart (Novomix), which has a better impact on postprandial glucose levels, and furthermore to implement a written algorithm for initiating and adjusting insulin dose. Thus the aim of our study was to evaluate the effect of this change in the routine insulin treatment guideline on birth weight and the prevalence of pregnancy related complications.
Materials and methods: The study population included all insulin treated women with GDM delivering in 2007 and 2009 at our hospital fulfilling the following inclusion criteria; GDM diagnosed and insulin treatment initiated < 34 weeks with the first HbA_{1c} below 6.5%, singleton pregnancies and at least two HbA_{1c} tests with ≥3 weeks' interval. Data was extracted from medical records. The insulin treatment was initiated and adjusted by a nurse the first 14 days and thereafter by an endocrinologist. The 2007-cohort received biphasic human insulin twice daily with individual start dose and adjustments while biphasic insulin aspart was initiated in the 2009-cohort with 0.3 IU /kg twice daily and thereafter adjusted according to a written titration guideline. The primary study outcome was the prevalence of large for gestational age (LGA) infants. Secondary outcome was a combined endpoint of pregnancy related complications including at least one of the following: preeclampsia, preterm delivery, birth complications, neonatal hypoglycaemia, respiratory distress syndrome and jaundice.
Results: The two cohorts were comparable with regard to baseline HbA_{1c} and HbA_{1c} prior to delivery (table). The total insulin dose was comparable, but the second cohort was diagnosed and initiated insulin treatment on average 10 days earlier and tended to be less overweight (NS). In the 2009-cohort offspring birth weight was significantly lower, evaluated by the Z-score as well as the prevalence of LGA. Moreover less pregnancy related complications were seen in the 2009-cohort (table).
Conclusion: The prevalence of large for gestational age infants and pregnancy related complications were lower after implementation of the new insulin treatment guideline as part of routine treatment. Whether this is due to the change in insulin treatment or other factors, as earlier initiation of treatment, remains speculative.

Clinical parameters before and after change in insulin treatment

	2007-cohort n=54	2009-cohort n=47
Pre-pregnancy BMI (kg/m^2)	31.5(7)	29.6(7)
Gestational age at GDM diagnosis(days)	190(43)	180(51)
HbA_{1c} at diagnosis of GDM (%)	5.7(0.4)	5.8(0.3)
Gestational age at delivery (days)	267(9)	269(8)
HbA_{1c}; last before delivery (%)	5.8(0.4)	5.8(0.4)
Insulin dose; last before delivery (IU)	52(40)	49(36)
Birth weight (g)	3516(441)	3297(491)*
Birth weight z-score (standard deviations)	0.8(1.4)	0.1(1.3)**
Large for gestational age	20(39%)	8(16%)*
Pregnancy related complication	20(37%)	9(19%)*

*Denotes p<0.05 and ** p<0.01 between groups. Mean(SD) or Number(%)

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Major congenital malformations in women with gestational diabetes mellitus: a systematic review and meta-analysis

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Background: Gestational diabetes mellitus (GDM) has been associated with a higher rate of congenital malformations, but the association is not universally accepted.
Aim: To perform a systematic review and meta-analysis on major congenital malformations (MCM) in GDM.
Methods: A MEDLINE search using the terms ((malformation OR outcome) AND (gestational diabetes) AND pregnancy)) was performed, limiting the search to the period January 2000 to December 2009. Selection criteria: 1) GDM and control populations are not openly biased; 2) Paper contains information on MCM in women with GDM and in the reference population; data on MCM in pregestational DM was not an inclusion criteria, but if included in the paper, information was recorded. Statistical analysis: Revman 5.0, with a fixed effect analysis method for meta-analysis.
Results: 1924 abstracts were retrieved, 108 full-text articles were revised and finally 9 cohort observational studies and 2 case control studies were included. In women with GDM, cohort studies displayed a high heterogeneity, precluding meta-analysis; in case-control studies, they had a higher rate of MCM (OR 1.40, CI95 1.22-1.62) in relation with the reference group. Women with pregestational DM had a higher rate of MCM vs reference group in both cohort (RR 2.34, CI95 2.02, 2.70) and case-control studies (OR 4.57, CI95 3.01, 6.95).
Conclusion: Infants of GDM mothers have a slightly higher increased risk of MCM, lower than that of women with pregestational DM.
Supported by: CIBER BBN

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Peripartum and gestational factors influencing neonatal hypoglycaemia in gestational diabetes: a prospective study

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Background: Most studies on neonatal hypoglycemia in GDM women only take into consideration gestational and neonatal parameters whereas the possible influence of peripartum factors hasn't been fully explored.
Objective: To evaluate peripartum, gestational and maternal factors influencing the development of neonatal hypoglycemia in infants of women with gestational diabetes.
Study design: Prospective observational study including all infants of GDM mothers born at our institution between October 2006 and February 2010. Data collected include maternal characteristics, gestational parameters (GDM treatment, weight gain, HbA1c), peripartum glycemic control (maternal capillary blood glucose (CBG) ketonemia, use of insulin) and CBG measurements in the newborn during the first 24 hours of life.
Results: A total of 183 infants were included with a mean weight of 3353 ± 505 grams and a gestational age of 39.2 ± 1.4 weeks. 30 newborns (16.4%) presented at least one CBG of less than 40mg/dl during the first 24 hours of life. The main maternal, gestational and peripartum characteristics in newborns with or without neonatal hypoglycemia are presented in Table 1. There were no significant differences between hypoglycemic and euglycemic infants in terms of neonatal weight, apgar scores at 5 and 10 minutes, umbilical cord artery and vein pH nor in the rate of small for gestational age. Macrosomia was more frequent in hypoglycemic newborns (21.2% vs 8%, p=0.024). Regarding maternal characteristics, Latin-American mothers were more likely to have infants with hypoglycemia than caucasian mothers (37% vs 17%, p=0.03). No significant differences were observed between other ethnicities (Pakistani, Moroccan, Asian).
Conclusions: Neonatal hypoglycemia does not seem to be influenced by glycemic control during labour. Other factors, such as ethnicity (latin-americans), insulin use during pregnancy and macrosomia, associate an increased risk for neonatal hypoglycemia.

Gestational and peripartum characteristics according to neonatal hypoglycemia

	Newborn CBG≥40mg/dl (n=153)	Newborn CBG<40mg/dl (n=30)	p value
Age (years), mean (SD)	32.7 (5.6)	33.3 (5.9)	0.572
Previous BMI (kg/m2) mean (SD)	27.1 (5.5)	26.2 (4.1)	0.417
Pregnancy weight gain (kg). mean (SD)	9.1 (4.2)	8.8 (2.7)	0.673
GDM treatment: diet/insulin (n/n)	84/44	11/14	0.041
Gestational age (week) mean (SD)	39.2 (1.4)	38.9 (1.4)	0.119
Preterm delivery, n (%)	6 (3.9)	3 (10)	0.167
Intrapartum mean maternal CBG, mean (SD)	99.7 (22.4)	94.9 (15.1)	0.269
Intrapartum maternal CBG> 130mg/dl n (%)	21 (13.7)	7 (23.3)	0.177
Intrapartum Ketosis, n (%)	20 (16.1)	2 (8)	0.372
Intrapartum insulin use, n (SD)	14 (9.2)	4 (13.3)	0.503

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In gestational diabetes mellitus, pregestational body mass index is an independent predictor of neonatal hypoglycaemia

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Background: Recently published data from the HAPO study reveal pregestational body mass index (BMI) as a predictor of cord blood C peptide; significance was not reached for the prediction of neonatal hypoglycemia (NH).
Aim: To assess pregestational BMI as a predictor of NH in women with gestational diabetes mellitus (GDM).
Methods: Database review of all GDM pregnancies (singleton and twins) attended in the Diabetes and Pregnancy Clinic of the center from 1st January 1981 to 31st December 2006. Outcome variable: NH defined as capillary blood glucose fulfilling Cornblath cut-off criteria in >=2 occasions in the first 48h of life. Screening for GDM was universal and used O'Sullivan test; diagnosis used NDDG criteria. We considered the following as potential predictors of NH: age, weight, height, BMI, weight increase during pregnancy, prior poor obstetric outcome, smoking habit, family history of DM, prior GDM/abnormal glucose tolerance, gestational age and glucose values at diagnosis, delay between diagnosis and treatment initiation, capillary blood glucose & HbA1c during pregnancy, insulin treatment, twin pregnancy and newborn sex; as potentially intermediate variables we considered maternal hypertension (chronic/pregnancy-induced), preterm birth, cesarean section, small and large-for gestational age newborns, abnormal Apgar and respiratory distress. Statistical analysis: bivariate analyses comparing characteristics of pregnancies with and without NH; logistic regression analysis (backward method) with NH as the dependent variable and aforementioned variables as potential predictors.
Results: During the study period, 2492 newborns of mothers with GDM were delivered (2228 singleton) and NH was observed in 3% of them. Mothers of NH newborns differed in a number of characteristics, one of them being prepregnancy BMI (24.45 vs 23.19 kg/m2, p <0.02). Logistic regression analysis identified pregestational BMI as a predictor of NH both when potentially intermediate variables were included (OR 1.293, CI 1.007, 1.660) or not (OR 1.359, CI 1.073, 1.721).
Conclusion: Pregestational BMI is an independent predictor of NH in this cohort of women with GDM.
Supported by: CIBER BBN

PS 104 Pregnancy - treatment

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Intensive glycaemic control in type 1 diabetic pregnancy: a comparison of continuous subcutaneous insulin infusion and multiple daily injection therapy

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Background and aims: Continuous subcutaneous insulin infusion (CSII) has been used in pregnancy and to date is thought to be non-inferior to a multiple daily injection (MDI) regimen. We reviewed a cohort of pregnant patients with type 1 diabetes mellitus (T1DM), and compared a cohort on CSII with a cohort treated with MDI, with the aim of assessing any difference in glycaemic control and pregnancy outcomes.
Materials and methods: We reviewed 507 women with TIDM who presented for antenatal care with our service over a 5-year period. There were 46 women treated with CSII and 461 treated with MDI. All subjects were asked to maintain daily 7-point profiles and these were reviewed weekly. Blood glucose measurement (BGM) targets were 5mmol/l or less pre-meals and 7mmol/l at one hour post-prandial. Maternal parameters including age, parity and weight were recorded. Glycaemic control represented by HbA1c in each trimester, attendance at a pre-pregnancy counseling clinic, and pregnancy outcome including Cesarean section rate, were recorded and differences were compared with Students' t-test and an odds ratio where appropriate.
Results: Women on CSII were older (35 ± 4 years Vs 31 ± 5 years, p<0.001), and booked earlier (6 weeks ± 2 Vs 8 weeks ± 5, p<0.05) to ante-natal diabetes care. Women treated with CSII had lower HbA1c levels at booking (6.5% ± 0.8 Vs 7.8% ± 1.4, p<0.001) and delivery (5.9% ± 0.3 Vs 6.3% ± 0.6, p<0.05). Cesarean section was recorded at a higher rate in those on CSII (67% Vs 46%, p<0.05). Birth weight did not differ between groups (3.6kg ± 0.6 Vs 3.5kg ± 0.8). There was no significant difference in peri-natal mortality between groups. Those treated with CSII were more likely to attend the pre-pregnancy service (40% of all pregnancies Vs 10%, OR 5.8, p<0.001). Women attending the pre-pregnancy service had lower HbA1c values at booking than those who did not attend (6.7% ± 1.4 Vs 7.8% ± 1.5, p<0.001). Patients treated with CSII attending the pre-pregnancy clinic had lower HbA1c values at booking than those treated with MDI (6.5% ± 0.7 Vs 7.0% ± 0.9, p<0.05), but there was no significant difference in HbA1c values at delivery between these two groups (6.3% ± 0.6 Vs 6.2% ± 0.7).
Conclusion: Women treated with CSII were more likely to book earlier to ante-natal services, to have attended pre-pregnancy services, and had better glycemic control than women treated with MDI. Cesarean section rates were higher in women treated with CSII despite similar birth weights at delivery in both groups. Peri-natal outcomes did not differ between groups. In our cohort CSII and MDI are both effective in improving maternal glycaemic control both pre-pregnancy and in pregnancy. CSII achieved lower HBA1c values by delivery. Both CSII and MDI are effective therapies for the management of T1DM in pregnancy.

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Conversion of pregnant patients with type 1 diabetes from multiple injection therapy to continuous subcutaneous insulin infusion in early pregnancy is safe and efficacious

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Background and aims: Adverse risks to mother and fetus in pregnant type 1 diabetic (T1DM) patients are attributed to poor maternal glycaemic control. Continuous subcutaneous insulin infusion (CSII) has not been proven to improve pregnancy outcome. Initiating CSII in early gestation due to poor glycaemic control or to replace long acting analogues may be associated with risk, as patients learn to use it. This study aimed to compare maternal glycaemic control, obstetric and fetal outcomes in diabetic pregnancies managed with CSII and multiple daily injections (MDI).
Materials and methods: In a retrospective case notes audit of 90 T1DM pregnancies (52 treated with CSII) between 2002 and 2009, we recorded HbA1c pre-conception and in each trimester; fetal ultrasound measurements; mode of delivery; age-corrected birth weight; APGAR scores and admission to neonatal intensive care unit (NICU). Primary comparison was between MDI and CSII. Secondary comparisons were between groups: CSII pre- (n=20) and post- (n=32) conception, MDI HbA1c <7.5% in trimester 1 (n=20) and MDI HbA1c >7.5% in trimester 1 (n=18). We compared MDI >7.5% with those converted to CSII due to poor control (n=8).
Results: CSII and MDI patients were comparable for age (31.9± 5.58 vs 29.9± 6.13 yrs p=0.1) and BMI (26.4± 4.04 vs 27.9± 5.92 yrs, p=0.2). CSII users were 84.6% Caucasian (vs 57.9% MDI, p=0.005), with longer diabetes duration (17.0± 7.05 vs 10.7± 7.98 yrs, p<0.001). CSII group achieved lower HbA1c in trimester 1 (7.0± 1.0 vs 7.6± 1.7%; p=0.03) and 2 (6.3± 0.7 vs. 6.8± 1.5%; p=0.04), with no significant difference in trimester 3 (6.2± 0.58 vs. 6.4± 1.0 p=0.3). Severe hypoglycaemia rates were not different. Fetal growth velocity was not different. There were more emergency Caesarean sections (42 vs 24% p=0.07) in MDI patients, with no differences in macrosomia, age-corrected birth weight centiles, or NICU admission. There were no differences in outcome between CSII pre-conception and MDI < 7.5%, nor between these groups combined and CSII post- conception. MDI >7.5% had significantly greater HbA1c in all trimesters, lower 5 minute APGAR score and greater NICU admission. Those converted to CSII post- conception due to poor control had lower HbA1c in trimester 1 (8.3± 0.67 vs 9.5± 2.0 p=0.06), 2 (6.9± 0.96 vs 7.8± 1.46 p= 0.15), and 3 (6.7± 0.83 vs 6.9± 1.06 p=0.6), than MDI >7.5%. This CSII converted group had higher 5 minute APGAR score (9.17± 0.76 vs 8.71± 2.08 p=0.09) and less NICU admission (38 vs 56% p=0.6).
Conclusion: Appropriately used, MDI can provide similar pregnancy outcomes to those using CSII for clinical indications. In those with raised HbA1c at booking, or requiring different basal insulin replacement regimens, initiation of CSII in early pregnancy is safe and efficacious leading to improved glycaemic control, and less neonatal intervention requirement.

Comparison between MDI and CSII in type 1 diabetic pregnancies

	MDI		CSII		P-value ANOVA
	HbA1c < 7.5% in trimester 1 (n=20)	HbA1c > 7.5% in trimester 1 (n=18)	Using CSII pre-conception (n=20)	Initiated CSII post-conception (n=32)	
1st trimester HbA1c mean (SD)	6.4 (0.61)	9.1 (1.50)	6.9 (0.80)	7.5 (1.08)	p<0.001
2nd trimester HbA1c mean (SD)	6.0 (0.92)	7.8 (1.45)	6.3 (0.67)	6.2 (0.72)	P<0.001
3rd trimester HbA1c mean (SD)	5.9 (0.61)	7.0 (1.06)	6.3 (0.51)	6.1 (0.63)	P<0.001
Corrected birth weight centiles mean (SD)	63.0 (30.10)	68.8 (36.68)	63.0 (33.75)	77.7 (28.86)	P=0.3
% Macrosomia	25	44	35	50	P=0.3
% emergency Caesarian section	40	44	20	23	P=0.4
APGAR 5 minute mean (SD)	9.5 (0.61)	8.7 (2.08)	9.7 (0.49)	9.5 (0.79)	P=0.05
% admitted to NICU	11	56	30	24	P=0.02
Congential malformations	1	1	0	1	P=0.4

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Glycaemic control and pregnancy outcomes in women with type 1 diabetes: a systematic review and meta-analysis comparison between lispro and regular insulin

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Background: There is limited evidence of the influence of insulin lispro (LP) vs regular (RI) on glycemic control and pregnancy outcomes in pregnancies of women with Type 1 diabetes mellitus (T1DM).
Aim: To perform a systematic review and meta-analysis on glycemic control and pregnancy outcomes in women with T1DM treated with insulin LP vs. RI since before pregnancy.
Methods: A Medline and EMBASE search were performed using the terms ((lispro OR Humalog OR (insulin analog) AND pregnancy) without any limit. Abstracts (and full papers when appropriate) were reviewed by two

independent researchers and differences set with a third one if needed. Inclusion criteria: T1DM, data on women treated with RI and LP since before pregnancy until delivery in the same paper, minimum number of 5 pregnancies in each group, information on at least one pregnancy outcome. Baseline characteristics, metabolic control during pregnancy and outcome data were extracted using predefined templates. Baseline characteristics were compared without performing a formal statistical analysis and outcome data were summarised with Revman 5.0.
Results: 267 abstracts were identified and 5 papers fulfilled inclusion criteria, all of them corresponding to observational studies. Women treated with LP or RI were similar in terms of age, BMI, diabetes duration, chronic hypertension and pre-pregnancy clinic attendance. Outcome data: HbA_{1c} were lower in patients treated with LP, at the beginning of pregnancy (mean difference (MD) -0.12; CI -0.49,-0.24) and in first (MD -0,33; CI -0.59,-0.08), second (MD -0.51; CI -0.83,-0.2) and third trimesters (MD -0.17;CI -0.38, 0.03). Gestational age at birth (MD 0.41; CI -0.04, 0.87), birthweight (MD 133.68; CI 19.35, 248) and the rate of large for gestational age newborns (LGA) (RR 1.35; CI 1.12-1.61) were higher in the LP group. No differences were observed in the rate of pregnancy induced hypertension, preeclampsia, diabetic ketoacidosis episodes, spontaneous miscarriages, interruptions, total abortions, caesarean section, preterm birth, macrosomia, small for gestational age newborns, stillbirth, neonatal and perinatal mortality, neonatal hypoglycemia and major malformations.
Conclusions: In relation with women with T1DM treated with RI, those treated with LP display similar baseline characteristics, achieve better glycemic control, deliver at a higher gestational age but birthweight and rate of LGA are higher.

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Exposure to teratogenic drugs and concomitant contraception in women of child-bearing age with type 2 diabetes: a population based cohort study

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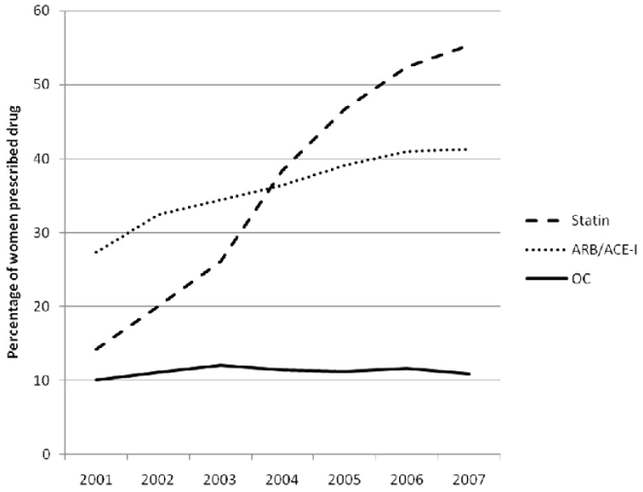
Background and aims: Twenty years after the St. Vincent declaration, pregnancy outcomes remain poor in women with diabetes. Perinatal mortality and congenital anomaly rates are four and two times that of the background population respectively. Preconception care, optimal glycaemic control and folate supplementation are known to reduce pregnancy risk in women with type 2 diabetes (T2DM). Despite this, many women remain poorly prepared for pregnancy and it is often unplanned. Since the introduction of the Pay for Performance in the UK in 2004, there has been more aggressive management of cardiovascular risk in people with diabetes. Some of the medications advocated for primary and secondary prevention of cardiovascular disease are potentially teratogenic. The aim of this longitudinal study was to identify changes in the prescription of cardiovascular medications and contraception for women of childbearing age with T2DM between 2000 and 2007 in the UK.
Materials and methods: Data were extracted from the General Practice Research Database (GPRD) for all women of child bearing age (14-49 years) with T2DM. Data were collected from seven consecutive annual time points between 2000 and 2007 and included details on age, body mass index (BMI), blood pressure, diabetes complications and prescription of statins, ACE-inhibitors, angiotensin-II receptor blockers (ARBs) and oral contraception (OC). Data were analysed in SAS 9.1.
Results: In 2001 the GPRD contained records of 1,195,600 female patients, of whom 1968 (0.16%) were aged 14-49 with T2DM. The study cohort size increased yearly to 5263 in 2007 (0.34% of GPRD female records). The proportion of women in each age group was consistent across the seven year period: 40% of women were aged 45-49 years, 28.5% 40-44 years, 16.5% 35-39 years, 8% 30-34 years, 3.5% 25-29 years, 2% 20-24 years and 1% 14-19 years. Of the 2007 cohort, 40% had an HbA1c >8%, 70% were obese and 27% had hypertension. Retinopathy was recorded in 11%, acute coronary syndrome in 1% and stroke or transient ischaemic attack in 1%. The use of statin therapy increased significantly in these women from 14% in 2001 to 55% in 2007 ($p<0.0001$ for trend) whilst OC use remained around 11% ($p= 0.8$ for trend) (see figure). In 2007, only 9.1% of women on a statin were also prescribed oral contraception.
Conclusion: Women of child bearing age with T2DM represent a high risk group. Despite increasing use of statins, ACE inhibitors and ARBs for primary and secondary prevention, OC prescription remains unchanged. This would suggest many women are at risk of pregnancy despite the prescription of potentially teratogenic drugs, further increasing the risk of poor pregnancy outcome. There is an urgent need to address preconception care in this rapidly expanding cohort of high risk women with T2DM.



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Low glycaemic index and hypocaloric diet therapy versus conventional approach in gestational diabetes/one abnormal value in pregnancy, after medical nutritional therapy failure

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Background and aims: We compared the efficacy of a low glycemic index and hypocaloric diet vs a conventional approach by insulin treatment in terms of maternal and fetal outcome, in GDM and OAV after medical nutritional therapy failure.
Materials and methods: Prospective Randomized open-label study (based on math criteria) exploring the two approaches. In 2007-2009, 97 pregnant women (63 GDM; 34 OAV; age 34,86±4,86; BMI 25,23 ±4,93 Kg/m²), were enrolled, at our diabetic and pregnancy unit. At the first visit (27,74± 7,25th gestational week) proper nutritional therapy was prescribed. In 18 women the total daily expenditure was measured by Metabolic Holter Body Media. Women with fasting glycemia ≤105mg/dl and postprandial glycemia ≤160mg/dl were assigned to "conventional medical nutrition therapy" ("A" Diet: about 30 cal/kg: 50-55%CHO; 20% protein; 25-30% lipids); for ethical reasons the women exceeding these cutoff BG levels, were directly treated with insulin injections (group EI). The elegible patients, were randomly shifted to a low glycemic index hypocaloric diet ("B" Diet: about 27 cal/kg: 45% CHO; 20% protein; 35% lipids) or to a classical approach with insulin (group I), when one week later, fasting BG was ≥90mg/dl and/or postprandial BG≥130mg/dl in ≥30% of each time point /week. Then, all the patients were strictly monitored, including daily self-control of ketonuria. Those belonging to group B were further shifted to insulin if the metabolic goals were not reached with low glycemic index and hypocaloric diet. STATISTICS**:** paired and Un-paired ***t***-test and χ^2.
Results: 22 subjects (23,17% of the population) kept being under diet A up the end of pregnancy (age: 34,95± 4,73; BMI 24,86±4 Kg/m²),17 (17,89%) were directly shifted to insulin for ethical reasons (34,4± 5 BMI 26,4 ±3,41 Kg/m²), whilst 30 women were randomized to B and 28 to I. The two groups were matched for the main anthropometric and metabolic indexes. 21 subjects of B (70% of B) achieved the metabolic goals until the delivery (age 35,9 ±4,3; BMI 24,48± 3,63 Kg/m²), whilst 9 needed to add insulin (30% of B) at 26,11±7,66th gestational week (age 33±2,87; BMI 32,42±6,63 Kg/m²). Significant differences were observed between B and BI for BMI ($p=0,003$). All groups are similar for type (CS=49; Spontaneous=37) and week of delivery (38,54± 1,69th gestational week). No differences were found for birth weight among B and I groups, but significant differences were observed between A and I as well between EI and I, with a total of 5 macrosomic births (EI=2; I=1;

A=2). In addiction levels for significance were observed for Ponderal Index ((kg/length m³) comparing A to I. No differences were observed for length (49,77± 2,24cm), APGAR 1' (8,7±0,62), APGAR 5'(9,7± 0,46), n° of hypoglycaemia (tot=5; 2 in EI and 3 in I), n° of hypocalcemia (2 in I).

Table 1. Maternal and fetal outcome

	Weight gain in pregnancy (kg)	Birth Weight (g)	Birth Ponderal Index (kg/m³)
A	12.14±3.87	3434.95±427.86	27,4±3,8
B	8.57±3.68	3209.44±380.75	25,4±2,1
BI	5.87±5.55	3326.66±412.00	27.06±3.03
I	9.63±3.93	3057.27±446.07	25.44±2.10
EI	13.61±6.11	3438.75±543.20	26.17±2.81

Weight gain in pregnancy (kg): A vs I p=0.03; A vs BI p=0.002; B vs EI p=0.003; BI vs EI p=0.006; BI vs I p=0.03; EI vs I p=0.01
Birth Weight (g): A vs I p=0.008; EI vs I p=0.02
Birth Ponderal Index (kg/m³): A vs I p=0.04

Conclusion: A low glycemic index and hypocaloric diet can be safely prescribed since the 1st visit in GDM and OAV.

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Effects of moderate physical activity on metabolic control in women with gestational diabetes

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Background and aims: Diet and exercise have been shown to be effective tools for prevention and treatment of all metabolic abnormalities. In spite of that, little information is available on the effect of lifestyle on glucose control in women with gestational diabetes (GDM). Therefore, we performed this study to evaluate whether moderate physical activity (PA) may improve metabolic control in women with GDM.
Materials and methods: After stabilization standardized diet based on pre-pregnant BMI, 32 GDM women (age 34.5±4.7 yrs, 31% primiparous, pre-pregnancy BMI 26.7±6.8 kg/m²) were invited to walk 30-min a day four times a week during pregnancy. At the beginning of 27±1 week of gestation, upon collection of anthropometric and metabolic parameters, a sensewear armband (SWA) was applied to all women for a 7-days monitoring of PA's intensity and duration, active energetic output and number of steps. During the same period, diet compliance was evaluated by a food frequency questionnaire and data elaborated using a computerized program (Metadieta®). All women were requested to measure capillary blood glucose 4 times a day during test week (fasting, 1hr after breakfast, after dinner and after lunch). Insulin treatment was allowed at the investigator's discretion.
Results: Study population was arbitrarily divided in active (n=16) and sedentary (n=16) women, based on median level of PA activity. There was no difference in age, parity, pre-pregnant BMI, and weight gain between the two groups. Active women had higher intensity (1.5±0.2 *vs.* 1.2±0.1 mets/day) and duration (102.5±57.2 *vs.* 47±20.4 minutes/day) of PA, greater active energetic output (466±235 *vs.* 244±99 Kcal/day) and higher number of steps (11,764±2,839 *vs.* 7,080±1,840) as compared to sedentary women (all p<0.05). There was no apparent difference in caloric intake (active: 1,895±477 *vs.* sedentary: 2,089±434 Kcal) and diet composition in the two groups (carbohydrates: 47.4±5.5% *vs.* 46.4±6.6%, fat: 35.5±2.5% *vs.* 36.1±2.7, proteins: 18.4±2.3% *vs.* 16.1±23%). No difference was found in blood glucose readings with the exception of lower 1-hr post-breakfast values in active women (5.9±0.8 *vs.* 6.6±0.1 mg/dl; p<0.04). Insulin therapy was deemed necessary in 12.5% of active women as compared to 50% of sedentary women (p<0.05), and when needed insulin requirement was lower in the former (0.1±0.5 *vs.* 0.3±0.2 IU/Kg/day; p<0.05). End-pregnancy HbA1c levels were lower in active than in sedentary women (4.8±0.3 *vs.* 5.5±0.2%; p<0.04). The former also higher serum HDL-cholesterol (77±11 *vs.* 63±14 mg/dl, p=0.006), while no differences were observed in total- and LDL cholesterol as well as triglycerides. The difference in PA had no effect on delivery time, percent of caesarean sections, and newborn health status.
Conclusion: Mild physical activity contribute to improve metabolic control in women with GDM reducing the number of women requiring insulin treatment and reducing insulin dose in those requiring it.
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Comparing pregnancy outcomes for intensive versus routine antenatal treatment of gestational diabetes based on a 75gram oral glucose tolerance test 2-hour blood glucose 7.8 - 8.9mmol/l

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Background and aims: The recent consensus guideline for gestational diabetes, from the HAPO group suggests a diagnostic 75 gram oral glucose tolerance test (OGTT) 2-hour blood glucose (2hr-BG) of 8.5mmol/l. Although HAPO and other trials show adverse outcomes for dysglycaemia in pregnancy, debate continues as to the efficacy of treating gestational diabetes (GDM) when the 2-hour blood glucose (2hr-BG) in the diagnostic 75 gm OGTT lies between 7.8 and 8.9 mmol/l. Our aim was to look at the effect of interventional treatment based on an OGTT 2-hr blood glucose 7.8 - 8.9mmol/l.
Materials and methods: A retrospective study covering 3.5 years between 2005 and 2008 between two clinics in teaching hospitals serving the same local population. Clinic A used, WHO (2-hr BG 7.8 mmol/l) and clinic B used, EASD (2-hr BG 9.0 mmol/l) diagnostic criteria for GDM. We compared the maternal and fetal outcomes for women whose 2-hr BG was between 7.8 and 8.9mmol/l. Clinic A patients were treated as GDM and managed intensively in the diabetes antenatal service and Clinic B were given dietary advice and managed conventionally.
Results: Demographics; women in clinic A N= 79 and Clinic B N=130 were well matched for age (33.2± 5.8 vs 33.1± 4.8 years) and BMI (28.6± 5.5 vs 27.2± 6.4). Values mean± sd. Centres were well matched for ethnic distribution and represented a multi-ethnic population: 39 vs 35% Black African and Caribbean: 14 vs 12% Asian: 47 vs 25% Caucasian and 0 vs 28% not documented. Screening values (mean±sd) showed no statistically significant difference: OGTT 2hr- BG (8.3± 0.32 vs 8.3± 0.34mmol/l) and HbA1c (5.39± 0.53% vs 5.44± 0.48%). Maternal Outcomes: women in clinic A had higher rates of induction (43% vs 21%), similar rates of caeasarian section (CS) (39%); with lower emergency CS rates (35% vs 74%). Fetal outcomes: women in clinic A had earlier gestational age for delivery (38.6± 1.3 vs 39.7± 1.9 weeks P<0.001); lower birthweight (3332± 504 vs 3556± 625grams P<0.05) and lower macrosomia rate (>4Kg 9% vs 25%).
Conclusion: Diagnosis of GDM with a OGTT 2hr-BG 7.8 - 8.9 mmol/l and treatment in a combined diabetes antenatal clinic is worthwhile with a decreased macrosomia rate and fewer emergency CS. There was an increased rate of induction, but no associated increase in CS.

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Metformin vs insulin in the treatment of gestational diabetes: impact of maternal pregestational BMI on birth weight and need for additional insulin during metformin

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Background and aims: In the previous MiG trial metformin and insulin treated mothers with gestational diabetes had infants with similar birth weights and rates of macrosomia. It can be hypothesized that with respect to neonatal weight, obese mothers could benefit more than non-obese mothers from metformin treatment vs insulin as metformin is known to induce less maternal weight gain, which in turn associates with fetal growth.
Materials and methods: We analyzed birth weight data from first 150 pregnancies in an ongoing randomized trial comparing metformin and insulin treatment of GDM. (Women with fasting P-glucose > 7.0 mmol/L or postprandial glucose > 11.0 mmol/L were not included in this trial.) In the present study we stratified the mothers by pregestational BMI 30 kg/m2 into obese and non-obese. Seventy-five mothers were included in both treatment groups. Mean maternal age (metformin vs insulin 31.8 vs 32.2 yrs), pregestational BMI (29.6 vs 28.5), glucose values in pretreatment OGTT or gestational weeks at delivery (39.3 vs 39.5 wks) did not differ between the two treatment groups.
Results: Birth weights tended to be higher (3658 g vs 3556 g, p = 0.19) in children of pregestationally obese compared with non-obese mothers (treatment groups combined). Birth weights were similar in metformin and insulin groups irrespective of maternal pregestational BMI. Need for additional insulin, defined as fasting P-glucose with metformin > 5.5 mmol/L and/or postprandial glucose > 7.8 mmol/L, was equally common in obese and non-obese metformin treated mothers. Rates of macrosomia were low in all study groups.

Conclusion: Compared with insulin, metformin is equally effective in the treatment of gestational diabetes in both obese (BMI > 30) and non-obese (BMI < 30) mothers. Additional insulin is needed in ca 20% of metformin treated mothers irrespective of obesity.

Birth weight and macrosomia stratified by treatment group and pregestational BMI

Group	n	additional insulin	birth weight, g	p for birth weight, metfo vs insulin	macrosomia, n (p metfo vs insulin)
Metformin, all	75	19 %	3629	0.41	4 (p=0.37)
Insulin, all	75		3567		1
BMI < 30, metformin	41	20 %	3601	0.35	1
BMI < 30, insulin	48		3519		1
BMI > 30, metformin	34	18 %	3663	0.94	3
BMI > 30, insulin	27		3652		0

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Two year's outcomes following the institution of combined ADIPS Guidelines for the management of diabetes in pregnancy

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Background and aims: In mid 2007, we reviewed our approach to the management of pregnant women with diabetes following a review of published guidelines. We noted that the recommendations for maternal and fetal monitoring and timing of delivery were not unified. For example North American authors suggest twice weekly non-stress CTG from 28-32 weeks. By contrast the Australian Diabetes in Pregnancy Society (ADIPS) Consensus Document states. Formal testing of fetal wellbeing (eg, cardiotocography, umbilical Doppler blood flow studies or biophysical profile) is not necessary in an otherwise uncomplicated pregnancy before 36 weeks gestation.
Materials and methods: In 2008 we adopted a more conservative approach to obstetric monitoring of diabetic women in pregnancy. This consisted of routine fetal heart rate (FHR) monitoring at 36 weeks and growth ultrasounds at 28, 32 and 36 weeks gestation. Insulin requiring women with unstable control were delivered at 39 weeks and those with optimal control were delivered at 40 weeks. Women with diet controlled gestational diabetes mellitus (GDM) were delivered by 41 weeks. The latter were followed throughout pregnancy in a routine antenatal clinic with review by obstetricians, diabetes educators, and dieticians. The aim of this review was to examine the outcomes of women managed with this approach during 2008 and 2009.
Results: The data are listed in the table 1. The one perinatal mortality was a fetus terminated at 20^{+6} weeks for Trisomy 13. There were fewer caesarean deliveries in those women with diet controlled GDM than in women taking insulin (34% vs. 48% P=0.005), fewer instrumental deliveries (9 vs. 22 P=0.028) and fewer admissions to SCN (1 vs. 16 P<0.001). There were no differences in birth weight, shoulder dystocia, admissions to NICU or 5 minute Apgar scores ≤7.
Conclusions: A more conservative approach in the management of diabetes in pregnancy is associated with good outcomes for a cohort of almost 400 women managed in our unit over a 2-year period. The majority of women in both groups delivered before the need for induction of labour.

Table 1. Pregnancy outcomes of the 390 women with diabetes in pregnancy.. Median±SE (range)

		Diet GDM (N=187)			Insulin Requiring (N=203)	
Delivery (weeks)		39.3 ± 0.21 (20.6-41.5)		39.1 ± 0.15 (24.5-40.6)		P=0.0005
Birth Weight (g)		3240 ± 47 (395-4560)		3325 ± 43 (661-4850)		NS
	Spont Lab (N=112).	Induction (N=50)		Spont Lab. (N=87)	Induction (N=68)	
Delivery (wks)	39.2 ±0.26 (25.5-41.3)	40 ±0.44 (20.6-41.5)	P=0.0027	38.5 ± 0.27 (24.5-40.1)	39.6 ± 0.13 (34.1-40.4)	P<0.001
Birth Weight (g)	3200 ± 67 (685-4560)	3347 ± 92 (395-4238)	P=0.03	3105 ± 69 (661-4390	3422 ± 62 (2020-4730)	P=0.0007

PS 105 Biomarkers in pregnancy

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Circulating vaspin levels are increased during pregnancy but shown no association with parameters of insulin sensitivity in women with gestational diabetes

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Background and aims: Visceral adipose tissue-derived serpin (Vaspin) is a novel adipokine that might be play a role in glucose metabolism. In humans, circulating vaspin levels were found to be increased in subjects with T2DM and to positively correlate with BMI and parameters of insulin sensitivity. In women with GDM, no differences in serum vaspin levels have been observed. However, acute glucose-induced changes under standardized conditions as well as effects of pregnancy itself on circulating vaspin levels remain to be elucidated.
Materials and methods: Plasma vaspin concentrations were measured in 20 pregnant women (10 GDM and 10 NGT) at 0, 30, 60 and 120 min of a 2h-75g-oral glucose tolerance test (OGTT) during gestational week 21-28 and three months after delivery by a commercially available ELISA kit (human Vaspin ELISA kit, Adipogen, Seoul, South Korea). Fasting insulin sensitivity was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR).
Results: At all timepoints of the OGTT, circulating vaspin concentrations were similar in women with GDM and NGT during pregnancy (1.54±0.86 vs. 1.33±0.76, p>0.05) as well as three months after delivery (0.26±0.42 vs. 0.32±0.69, p>0.05). Post-partum, plasma vaspin levels decreased significantly in both groups (p<0.01, respectively), however, to similar extent (p>0.05). Only in women with GDM, circulating vaspin was significantly decreased 60 and 120 min after glucose ingestion during pregnancy (p<0.01). Plasma vaspin concentrations correlated signficantly with glutamic-oxaloacetic transaminase during pregnancy (r=-0.49, p=0.03) as well as serum estrogen levels (r=-0.51, p=0.03), sexual hormone-binding globuline (r=-0.46, p=0.05) and serum creatinine (r=0.53, p=0.02) three months after delivery. No association between circulating vaspin levels and parameters of insulin sensitivity including HOMA-IR, fasting glucose, insulin or C-peptide levels have been observed.
Conclusion: In contrast to previous studies, we found no association between parameters of insulin sensitivity and circulating vaspin concentrations in women with GDM. Interestingly, following glucose loading plasma vaspin concentrations decreased only in women with GDM. Additionally, pregnancy seems to result in an elevation of circulating vaspin.

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Plasma chemerin concentrations in relation to parameters of insulin sensitivity in women with gestational diabetes

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Background and aims: Chemerin, a novel adipokine, is a chemoattractant protein with several functions in innate and adaptive immunity and implicated in the regulation of glucose homeostasis. Previously, chemerin was found to enhance insulin-stimulated glucose uptake in 3T3-L1 adipocytes, to induce insulin resistance in skeletal muscle and to exacerbate glucose intolerance in mice, when administered exogenously. In humans, circulating

chemerin levels are increased in insulin-resistant states, including obesity, polycystic ovaries syndrome or non-alcoholic fatty liver disease, and to decrease following bariatric surgery. Further, insulin and metformin were suggested to regulate circulating chemerin concentrations. The objective of the present study was to evaluate whether GDM is associated with changes in plasma chemerin concentrations.
Materials and methods: Plasma chemerin concentrations were measured in 20 pregnant women (10 GDM and 10 NGT) at 0, 30, 60 and 120 min of a 2h-75g-oral glucose tolerance test (OGTT) during gestational week 21-28 and three months after delivery by a commercially available ELISA kit (R&D Systems, Minneapolis, MO). Fasting insulin sensitivity was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR).
Results: No differences in circulating chemerin levels at any timepoint of the OGTT were observed between GDM and NGT during pregnancy or after delivery (p>0.05). However, the decrease in plasma chemerin concentration between 0 min and 30, 60 or 120 min of the OGTT was more pronounced in women with GDM during pregnancy (p<0.05, respectively) but not postpartum. Fasting plasma chemerin levels correlated with HOMA-IR (r=0.54, p=0.01), fasting insulin (r=.0.53, p=0.02) and fasting C-peptide levels (r=0.69, p<0.001), HDL-cholesterol (r=-0.47, p=0.04), CRP (r=0.58, p=0.008) and usCRP (r=0.65, p=0.004) during pregnancy and with HOMA-IR (r=0.54, p=0.02), fasting insulin (r=0.56, p=0.02) three months after delivery. The decrease in plasma chemerin following delivery (p<0.05, respectively) tended to be higher in women with GDM (p=0.08).
Conclusion: Our study shows that plasma chemerin levels correlated with parameters of insulin sensitivity and inflammation also in women with GDM. However, no significant differences have been found in women with GDM as compared to pregnant, normal-glucose tolerant women. Thus, the potential role of chemerin in the pathogenesis of GDM remains to be determined.

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Circulating endothelial progenitor cells are reduced in pregnant women with abnormalities of glucose tolerance

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Background and aims: Bone marrow-derived endothelial progenitor cells (EPCs) contribute to support vascular integrity. A role for EPCs has been claimed also in development and maintenance of the vasculature during pregnancy whose cardiovascular adaptation sustains the developing foetus. Gestational diabetes is associated with systemic endothelial dysfunction, but only a very few data about EPCs in pregnancies complicated by diabetes are available.
Materials and methods: We quantified circulating EPCs in pregnant women with abnormalities of glucose tolerance undergoing a three-hour, 100-g oral glucose tolerance test (OGTT). Insulin sensitivity and β-cell function indexes were derived from fasting steady-state and from the OGTT. EPCs (CD34+KDR+CD133+ cells) were quantified by three colours flow cytometry in 23 women with normal glucose tolerance (NGT), 18 women with gestational impaired glucose tolerance (GIGT) - defined as a single abnormal value on OGTT - and 24 subjects with gestational diabetes mellitus (GDM). Tests were performed at 27±3.2 weeks of gestation.
Results: Women with GDM, GIGT and NGT were comparable for age, family history of diabetes, pre-pregnancy body weight, BMI, incremental gestational body weight and blood pressure. GDM showed mean glycemic response higher than women with GIGT and NGT. AUCGluc (p<0.0001) and AUCIns (p=0.06) increased from NGT to GIGT to GDM. Insulin sensitivity indexes, ISIcomp and OGIS, reduced progressively in women with NGT to those with GIGT and GDM (ISIcomp: 4.92±2.05, 4.43±2.68, and 3.35±1.87, respectively, p<0.05; OGIS 387±53, 357±70, and 316±79 mg/min/m2, respectively, p<0.005). Finally, the ISSI index that estimates insulin secretion with respect to prevalent insulin sensitivity, was higher (p<0.0001) in women with NGT. The number of circulating CD34+ cells resulted similar in the three groups (NGT: 353.7±165.9; GIGT: 417.2±242.5; GDM: 423.3±220.0 cells/106 events), while circulating EPCs differed among women with GDM, GIGT and NGT (p=0.0172). Namely, EPCs were significantly higher in NGT (55.6±57.8) when compared to both GIGT (26.5±19.6; p=0.018), and GDM (26.5±20.4 cells/106 events; p=0.011), with no differences between GIGT and GDM. EPCs were inversely correlated with age (r=-0.26, p=0.04), as well as with 1-h (r=-0.30, p<0.02) and 2-h post-load plasma glucose (r=-0.36, p<0.005), and AUCgluc (r=-0.37, p<0.005), but not with insulin levels or AUCins. A weak positive correlation was also observed between EPCs and ISSI (r=0.25, p<0.05). Finally, no associations have been shown between EPCs and fasting (HOMA%S: r=-0.13, p=0.32) or dynamic indexes of insulin sensitivity (ISIcomp: r=0.01, p=0.95; OGIS: r=0.15, p=0.24). In a multiple linear regression model, only age (p=0.021) and AUCgluc (p=0.027) remained significantly associated with EPCs count.
Conclusion: Alterations of glucose tolerance during pregnancy, other than affecting insulin sensitivity and secretion, seems to act as the triggering factor for the EPC depletion.

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A high level of prorenin in early pregnancy is associated with development of preeclampsia in women with type 1 diabetes

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Background and aims: Preeclampsia is characterised by abnormal placentation in early pregnancy, maternal systemic endothelial dysfunction and perturbation of the renin-angiotensin-system. Levels of semicarbazide-sensitive amine oxidase (SSAO) have been shown to be positively associated with angiotensin converting enzyme (ACE) activity and are elevated in patients with diabetes. SSAO is implicated in the pathophysiology of diabetic late complications and may be a marker of endothelial dysfunction and preeclampsia. We investigated whether SSAO and components of the renin-angiotensin system in early pregnancy are associated with development of preeclampsia in women with type 1 diabetes.
Materials and methods: Observational study of 107 consecutive pregnant women with type 1 diabetes for median 16 years (range 1-36) and HbA_{1c} 6.6% (4.9-10.5) in early pregnancy. At 8, 14, 21, 27 and 33 weeks blood samples were drawn for measurements of prorenin, renin, angiotensinogen, ACE and SSAO; HbA_{1c}, blood pressure and urinary albumin excretion (UAE) were recorded. Preeclampsia was defined as blood pressure >140/90 mmHg (two measurements) accompanied by UAE ≥300 mg/24h later than 20 weeks.
Results: Preeclampsia was recorded in nine women (8%) characterised by a longer duration of diabetes (median 20 years (range 10-32) vs. 16 (1-36), p=0.04) and higher levels of SSAO at 8 weeks (592 (372-914) mU/L vs. 522 (264-872), p=0.04, normal range 352±102 mU/l) compared with women without preeclampsia. At 8 weeks prorenin levels tended to be elevated in women with subsequent preeclampsia (136 (50-296) mU/L vs. 101 (21-316), p=0.06, normal range 88 to 390 mU/L), whereas levels of renin, angiotensinogen and ACE were comparable (p=0.90, 0.73 and 0.91, respectively). From 8 to 33 weeks prorenin levels decreased slightly and similarly in women with and without preeclampsia, but throughout pregnancy, prorenin levels remained 28% higher (p=0.0096) and SSAO levels 16% higher (p=0.04) in women developing preeclampsia whereas levels of renin, angiotensiongen and ACE were comparable between the two groups (p=0.49, 0.75 and 0.53, respectively). In univariate logistic regression analyses of continuous variables, development of preeclampsia was associated with prorenin levels at 8 weeks (odds ratio 4.4 [95% confidence interval 1.5-13.0], p=0.007), UAE (≥30 vs. <30 mg/24h) at 8 weeks (3.1 [1.3-7.7], p=0.01), SSAO levels at 8 weeks (1.8 [1.1-3.1], p=0.01) and duration of diabetes (1.1 [1.003-1.2], p=0.04). In multivariate logistic regression analysis, the only independent predictor of preeclampsia was prorenin levels at 8 weeks (4.4 [1.5-13.0], p=0.007), i.e. an increase of prorenin of 100 mU/L results in a 4.4 times higher risk of developing preeclampsia.
Conclusion: In women with type 1 diabetes, a high level of prorenin in early pregnancy was associated with development of preeclampsia. Whether these changes in the renin-angiotensin system mainly reflect maternal susceptibility present before pregnancy or represent changes secondary to abnormal placentation remain speculative.

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Relationship between perinatal outcomes and thyroid-peroxidase antibodies (TPO) in a cohort of pregnant women with gestational diabetes (GD)

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Background and aims: The prevalence of TPO antibodies in pregnant women ranges between 6-10%, in line with what is found in the average population. Various non-organ specific auto-antibodies have been found associated with GD. The presence of TPO antibodies has been independently related to increasing rates of spontaneous abortion, preterm delivery, and it has recently been demonstrated that in the first trimester there is a risk factor of perinatal death. The aim of this study was to evaluate the effect of maternal autoimmunity on perinatal outcomes and on maternal morbidities.
Materials and methods: GD was diagnosed in a cohort of 1501 pregnant women without pregestational thyroid dysfunction, gestational age in the first visit >12 week, singleton pregnancy and availability of TPO antibodies titer
Results: 341 patients (22.72%) were TPO antibodies positive (>11.9 U/ml). There were no differences between the groups of pregnancies according to TPO antibodies status in mean maternal age [32.6 (SD: 4,2) vs 32.7 (7.5) years], BMI [24.9 (4.7) vs 24.9 (6.6) kg/m^2], frequency of nulliparities [163 (47,8) vs 553 (47,6)], percentage of insulinizations [61 (17,8) vs 72 (14,8) %] and average of A1c hemoglobin [4,24 (0,47) vs 4,26 (0,53) %]. There were no significant differences as regards the prevalence of the hypertension (pregestational + gestational) [18 (5,2) vs 56 (4,8) %], preeclampsia [4 (1,1) vs 16 (1,3) %] among the women with positive TPO antibodies and the group with negative TPO antibodies. We demonstrated a higher frequency of recurrent miscarriages (≥ 3 abortions) among pregnant GDM with TPO+ *vs* pregnant GDM with TPO- [12 (1.35) vs 14 (1.2), RR: 2,61: CI 95: 1.22-5.60)].There were no differences between the percentage of preterm deliveries (<34 weeks of pregnancy [7 (2,05) vs 12 (1,03) %] nor in <32 weeks [3 (0,87) vs 6 (0,51)]). Perinatal mortality was equiparable between both groups of women [1 (0,29) vs 2 (0,17)]. There were no differences between the mean weight of neonates, no differences in the prevalence of big for gestational age [14 (4,1) vs 53 (4,5) %] nor in the neonates with weight <2500 g [13 (3,8) vs 36 (3,1) %].
Conclusion: Considering the high prevalence of positive TPOantibodies in women with GD and the increasing risk of developing postpartum dysfunction in this group, a screening of thyroid function during pregnancy and a postpartum follow-up are recommended for these women. This study confirms the correlation between positive TPO during the pregnancy and higher frequency of recurrent miscarriage. In our cohort, thyroid autoimmunity is not related with an adverse effect on perinatal outcome in the babies born to patients with GD.

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The influence of pregnancy and gestational diabetes on serum levels of osteocalcin, osteoprotegerin and RANKL

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Background and aims: Osteoprotegerin (OPG), a soluble tumor necrosis factor-like protein secreted by osteoblasts, exerts an inhibitory effect on osteoclastic bone resorption by binding and neutralizing the receptor activator of nuclear factor-κB ligand (RANKL). Besides, OPG has other biological functions, including anti-inflammatory and anti-apoptotic effects. Recent studies suggest also that osteocalcin, another osteoblast - derived protein acting locally on bone formation, increases beta-cell proliferation and insulin secretion and improves insulin sensitivity. Conflicting results concerning serum osteocalcin and osteoprotegerin concentrations have been obtained in patients with obesity, type 2 diabetes,as well as gestational diabetes (GDM). The aim of the present study was to evaluate possible changes in serum osteocalcin, OPG and RANKL levels in pregnant women with normal glucose tolerance (NGT) and GDM.
Materials and methods: Serum osteocalcin, OPG and RANKL levels were measured, using enzyme-linked immunosorbent assays, in 47 patients with GDM and 35 healthy pregnant women in the 3rd trimester of pregnancy and 12 weeks postpartum.
Results: There were no significant differences in the concentrations of osteocalcin, OPG and RANKL between the women with GDM and NGT both before (7.8 [5.6-10.5] ng/ml vs 9.5 [5.1-12.7] ng/ml, 6.3 [4.9-7.2] pmol/l vs 5.4 [3.8-7.2] pmol/l and 0.22 [0.14-0.59] pmol/l vs 0.31 [0.12-0.46] pmol/l, respectively) and after delivery (22.1 [16.5-29.6] ng/ml vs 23.1 [17.4-29.7] ng/ml, 4.2 [3.7-5.2] pmol/l vs 3.9 [3.4-4.5] pmol/l and 0.26 [0.13-0.48] pmol/l vs 0.31 [0.10-0.62 pmol/l, respectively). Twelve weeks postpartum serum osteocalcin concentrations increased (p<0.0001), whereas OPG levels decreased significantly (p<0.0001) in both groups of patients in comparison with the pregnant state. In the whole group studied there was a significant correlation between osteocalcin levels and gestational age (R=0.30, p=0.02). In healthy pregnant women osteocalcin levels correlated with gestational age (R=0.45, p=0.04) and BMI values (R=0.83, p=0.04), while OPG concentrations were related to glucose levels 60 and 120 min after glucose load (R=0.57, p=0.005 and R=0.50, p=0.01, respectively). In the same group there was also a negative correlation between RANKL concentrations and gestational age (R=-0.42, p=0.04). No associations of the parameters studied with insulin and HOMA-IR were noted.
Conclusion: It could be hypothesized that lower osteocalcin levels may be related to decreased insulin sensitivity and increased fat mass in pregnant women. On the other hand, increased OPG concentrations, possibly due to elevated estrogen levels during pregnancy, may play protective roles including an inhibition of excessive bone resorption and anti-inflammatory actions. No effect of GDM on serum osteocalcin and OPG/RANKL system was found in the present study.
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Vitamin D deficiency and isolated fasting hyperglycaemia in pregnancy

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Background and aims: The relation between vitamin D deficiency and Gestational Diabetes Mellitus (GDM) has been rarely addressed in the literature with conflicting results. The aim is to determine the association between maternal serum 25(OH)D and glucose metabolism in Caucasian pregnant women.
Materials and methods: In a prospective study 157 pregnant women aged 31.2±5.8 years underwent a 100g OGTT in the third trimester of pregnancy, during which serum 25(OH)D, PTH, Ca, and P concentrations were also measured. For GDM diagnosis the ADA 2000 criteria were used. For 25(OH)D deficiency a cut-off point of 20ng/ml was chosen. Age, height, prepregnancy weight, BMI and blood pressure (BP) were recorded. Indices of insulin secretion and sensitivity were calculated. 25(OH)D was converted to its natural logarithm(Ln).
Results: 25(OH)D deficiency was found in 88 out of 157 pregnant women(56%). There was no difference in mean serum 25(OH)D between Normal (n=95, 20.2±7.0ng/ml), Isolated Hyperglycaemia (n=30, 22.1±7.7) and GDM (n=32, 19.4±8.3) women. Ln-25(OH)D was negatively correlated with fasting plasma glucose (r=-0.174, 95% CI 0.020-0.326, p=0.029). The relationship remained significant after adjustment for BMI, age, gestational age and seasonal variation. The percentage of isolated fasting hyperglycaemia (Glu0 ≥ 95mg/dl) was significantly increased in the subgroup of pregnant women with vitamin D deficiency (<20ng/ml) compared to the counterparts with 25(OH)D>20ng/ml (27.3% vs7.2% respectively, p<0.01). The odds ratio of isolated fasting hyperglycaemia in women with 25(OH)D<20ng/ml was 4.8 (95% CI 1.7-13.3). Also, a weak but significant correlation was found between Ln-25(OH)D and indices of insulin resistance (QUICKI: r=0.191, p<0.05, HOMA-IR: r= -0.192, p<0.05). There was no correlation between Ln-25(OH)D and indices of insulin secretion. Further, we confirmed the expected negative correlation of Ln-25(OH)D with PTH (r=-0.446, p<0.001), and also with systolic (r=-0.203, p=0.011) and diastolic BP(r=-0.238, p=0.003).
Conclusion: We found an independent negative correlation of 25(OH)D with fasting glucose in pregnant women. Furthermore 25(OH)D deficiency was significantly associated with increased risk for isolated fasting hyperglycaemia. Finally 25(OH)D deficiency is common in Greek pregnant women.

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Markers oxidative stress and antioxidant status in women with late-onset gestational diabetes mellitus

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Background and aims: The relationship between late-onset gestational diabetes mellitus [GDM] and oxidative stress is not well known and the importance of the oxidant/antioxidant equilibrium in the clinical evolution and complications of late-onset GDM require elucidation. The aim of the present study was to evaluate the relationships between maternal serum levels of markers of oxidative stress in women with late-onset GDM which potentially may have considerable clinical implications in the pathogenesis and/or the evolution of GDM.
Material and methods: We performed a nested case-control study within a sample of a total of 126 pregnant women (63 with GDM, 63 controls), between the 24th and 29th week of gestation. Both groups were analyzed for demographic data, perinatal and obstetrics results and the levels of the markers oxidative stress and antioxidants status, that were measured in serum or plasma using a commercial kit (Cayman Chemical, Ann Arbor, MI, USA).
Results: In the univariate analysis, control *versus* patient results were: maternal age 30.52±4.05 vs. 31.43±4.4 years (p=0,1); pre-gestational body mass index [BMI] 23.31±4.2 vs. 27.13±4.6 kg/m^2 (p=0.001); weeks at delivery 39.2±3.05 vs. 38.9±1.8 (p=0.09); Caesarean delivery 12.5 vs. 43% (p=0.004); macrosomia 4 vs. 9.4% (p=0.6); lipoperoxides [LPO] 2.06±1.00 vs. 3.14±1.55 µmol/mg (p=0.001); catalase 3.23±1.41 vs. 2.52±1.3 nmol/min/ml (p=0.03); superoxide dismutase [SOD] 0.11±0.04 vs. 0.08±0.01 U/ml (p=0.0003); glutathione peroxidase [GPX] 0.03±0.006 vs. 0.025±0.006 nmol/min/ml (p=0.01); glutathione reductase [GSH] 0.004±0.002 vs. 0.004±0.004 nmol/min/ml (p=0.9); glutathione transferase [GST] 0.0025±0.0012 vs. 0.0027±0.00017 nmol/min/ml (p=0.7). Multivariate analysis that was performed using non-conditional logistic regression showed catalase having a protective effect against (OR=0.39, p=0.006) and LPO carried a significant risk for GDM (OR=2.44, p=0.034).
Conclusion: These data suggest an increase in oxidative stress and a decrease in antioxidative defence in women with late-onset GDM and, as such, may have considerable clinical implications in the pathogenesis and/or the course of the pregnancy in these patients.

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Oxidative stress may stay elevated after gestational diabetic and even healthy pregnancies

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Background and aims: The number of pathological and moreover healthy pregnancies increases the risk of cardiovascular morbidity. Parous women with no complicated births have a 1.95-fold higher cardiovascular disease prevalence compared to nulliparas. Among women with one or more pregnancy complications, cardiovascular disease prevalence is 2.67 times higher. It was shown that increased oxidative stress can be observed during the course of normal pregnancy. Gestational diabetes mellitus (GDM) is associated with a pronounced degree of oxidative stress in placental and umbilical cord tissues and also in the plasma of the mother and the newborn. It is also known that elevated levels of oxygen and nitrogen derived reactive species are major contributors in the development of cardiovascular morbidities. Our aim was to examine the level of possibly persisting oxidative stress and its correlation to other known cardiovascular risk factors after pregnancy.
Materials and methods: Serum total peroxide level was measured in healthy volunteers three years following healthy (n=20), moderate GDM (carbohydrate-restricted diet, n=30) and severe GDM (insulin treatment, n=12) pregnancies. Controls were age and BMI matched males (n=10) and nulliparous women (n=14). In order to characterize their carbohydrate metabolism fasting glucose, HbA1c levels were measured and oral glucose tolerance test (oGTT, 75g) was performed. Serum glucose and insulin levels were determined in every 30 minutes, area under the curve was calculated. The following clinical parameters were also gauged: inflammatory markers, lipid profile, liver and kidney function, thyroid and sex hormones. Serum total peroxide level was measured in fasting conditions and 2hours following oGTT.
Results: The oGTT did not change the peroxide level in any study group. In fasting conditions the peroxide level of nulliparous women and men was similar. Previous healthy pregnancy significantly elevated (586.2±66.8 vs. 332.3±34.5umol/l, p<0.05) the peroxide level and previous moderate GDM did not increased it further on (657.2±46 umol/l). However severe GDM resulted in additional significant increase (951±147.7 umol/l p<0.05, vs. nulliparous, moderate GDM). Factors that may influence peroxide level were analyzed in multivariate regression model, in order to select significant variables stepwise method was used. According to the model peroxide level is significantly influenced by CRP (33.9umol/l, p<0.05) SHBG (2.7umol/l, p<0.05), total serum protein (22.8µmol/l, p<0.05) and the number of pregnancies (b=78.6 umol/l, p<0.05) (r2=0.46). The number of pregnancies significantly correlates with CRP (Pearson correlation: 0.32, p<0.05), which shows that the effect of previous pregnancy on peroxide level is partially due to the increase in CRP level.
Conclusion: According to our results the elevated oxidative stress that can be measured during pregnancy can be still observed three years after delivery. The level of oxidative stress is only altered by previous severe GDM, but not moderate GDM. Immunological processes may play important role in this phenomenon. The elevated level of oxidative stress may contribute to the increased cardiovascular morbidity of child-bearing women.
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PS 106 Pregnancy - pathophysiology

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Metabolic variables (fructosamine, HbA_{1c}) as predictors and pathophysiologic markers of gestational diabetes mellitus and diabetic fetopathy

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Background and aims: The current therapeutic strategies to reduce macrosomia rates in GDM have focused on normalization of maternal glucose levels throughout pregnancy. This study compares HbA1c, fructosamine, a fructosamine/total protein ratio and an index consisting of fructosamine/ total protein x 1-h-postprandial glucose/100 in regard to their possibility as predictors and pathophyiological markers of GDM and infant´s macrosomia.
Materials and methods: A total of 715 pregnant women underwent an oral glucose tolerance test and were grouped in four categories according to their glucose tolerance and infant´s birthweight. 85.9% (307) women with NGT had normal weight children, while 13.9% (50) presented with macrosomia. For women with GDM, 89.1% (319) gave birth to a child with normal weight, while 39 (10.9%) delivered macrosomic infants. In all pregnant women 89 women (~12%) presented with fetal macrosomia. Subsequent HbA1c, fructosamine, ratio and index were compared in regard to their accurancy for the diagnosis of GDM and macrosomia by ROC curves.
Results: We found a poor correlation of fructosamine with oGTT data, although the ratio was developed as a more sensitive form than single serum fructosamine concentration. According to correlations between HbA1c and oGTT values, we can conclude that HbA1c measurements have low sensitivity as predictive markers for diagnosis of GDM as well as for macrosomia, both in NGT and GDM subjects.Due to strict glycemic control (blood glucose fasting < 91 mg/dL, 1 hour postprandial < 131 mg/dL) there was no difference in the rate of macrosomia. In all women birthweight was associated with BMI, fasting plasma glucose, calculated ratio, HbA1C, weight and gestational age (all data preconceptional). For women with NGT only obesity seems to have an impact on infants´ birthweight, while for women with GDM additional metabolic parameters apart from obesity affect fetal growth. Ratio was the only variable able to differentiate within the GDM subgroups and was the best predictor for birthweight of infants. On the other hand the index achieved a satisfying sensitivity in regard of diagnosis of GDM. For women with macrosomic infants again the index was superior in detecting GDM.
Conclusion: In conclusion, women with GDM feature higher levels of HbA1c and fructosamine as well as higher index values. Due to overlap of NGT and GDM groups none of these parameters can offer an alternative to standard oGTT screening procedures but could be used as additional parameters for confirmation of impaired glucose tolerance and prediction of worse outcome. It remains the central target to modify the traditional GDM screening process in order to provide a test that should be more reliably detecting those women at risk of developing GDM during pregnancy and/or delivering a macrosomic infant despite negative testing for GDM.

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The effect of weight gain on gestational diabetes mellitus

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Background and aims: To evaluate the association between gestational diabetes mellitus (GDM) and weight gain during pregnancy.
Materials and methods: A prospective cohort study of 614 consecutive gravid patients, screened for GDM using 50-gram glucose challenge test (GCT) between June - December 2009. The pregnant women were divided into 4 groups according to their pre-pregnancy body mass index (BMI). Group I, II, III and IV constituted when the BMI <18.5 kg/m^2 (n=16), 18.5-24.9 kg/m^2 (n=455), 25-29.9 kg/m^2 (n=122) and >30 kg/m^2 (n=21), respectively. All the pregnant women were also evaluated in terms of their weight gain during pregnancy and these cases were recruited in 3 groups as low, ideal and high weight gained groups.
Results: Overall, a positive GCT result was identified in 109/614 (17.8%) women. GDM was further diagnosed in 12/614 (1.95%) of subjects. While the prevalence of GDM in patients with a normal pre-pregnancy weight was 1.31%, in over-weight and obese patients it was 3.28% and 9.52% respectively. The frequency of the GDM significantly increases in obese patients at the pre-pregnancy period compared to the ones with normal BMI. The cases of group II and group III showed statistically significant positive results of 50 g GCT when they had excess weight gain compared to the ones whose weight gain stand in a normal range.
Conclusion: If the women with normal BMI gain more than the recommended weight range during their pregnancy, they should be regarded as having risk for GDM.

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The effect of lactation on glucose and lipid metabolism in women with prior gestational diabetes

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Background and aims: Lactation confers health benefits to women with a history of gestational diabetes (GDM). Breastfeeding improves glucose tolerance in the early postpartum period, but it is unclear whether future risk of metabolic alterations, like type 2 diabetes, is reduced. The aim of this study was to investigate the effect of lactation, three years after pregnancy, on glucose and lipid metabolism in women with prior gestational diabetes.
Materials and methods: A population of women with prior gestational diabetes, according to Carpenter and Coustan Criteria, was evaluated with comparison of results for lactating versus nonlactating women. A total of 81 women participated (62 breastfeeding [BF] and 19 nonbreastfeeding [<4 weeks , nonBF]). Each woman completed a 75-g oral glucose tolerance test (OGTT) to analyze glucose tolerance, insulin sensitivity / resistance and b-cell function. Fasting serum was used to investigate lipid profile (total cholesterol, high-density lipoprotein [HDL] cholesterol, low-density lipoprotein [LDL] cholesterol, and triglycerides), apolipoprotein B, apolipoprotein A1, homocysteine, fibrinogen, hs- CRP, uric acid, microalbuminuria. STATISTICS: paired and Un-paired t-test, Mann-Whitney and χ^2 tests were used, as appropriate.
Results: The mean (+/- standard deviation) maternal age (37.1 +/- 4.6 versus 37.4 +/- 4.9 years), body mass index (26.3+/- 5.6 versus 26.4 +/- 5.3 kg/m2), and parity (1.9+/- 0.8 versus 1.7 + 0.8) , were not different between the lactating and nonlactating women. No effect was visible on glucose tolerance, HOMA-IR and other b-cell function indexes as well as hs- CRP (not significantly lower in non BF), uric acid, total cholesterol, HDL and LDL cholesterol. Levels for significance were only found for: HOMA-IS (BF1.0 +/- 0.7 vs non BF 0.6 +/- 0.4, p = 0.04), Triglycerides (BF 83.8 +/- 46.7 vs non BF 123.2 +/- 94.0 mg/dl, p = 0.02).
Conclusion: Breastfeeding had no effects on glucose tolerance status three years after the delivery of women with prior GDM.

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Association of fatty liver index (FLI) with parameters of insulin sensitivity in women with prior gestational diabetes

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Background and aims: Most of the important risk determinants of the metabolic syndrome, including obesity, insulin resistance, glucose intolerance, dyslipidemia and hypertension are present in women with prior gestational diabetes (pGDM) and indicate their high risk for developing type 2 diabetes and cardiovascular disease (CVD) in later life. Fatty liver (FL) is associated with insulin resistance, in particular in the liver, and with CVD. The aim of the study was to evaluate the relationship between pGDM, presence of FL and insulin resistance.
Materials and methods: A cross-sectional analysis was performed in 62 pGDM women and 28 women with normal glucose tolerance during pregnancy (NGT) until 3 months after delivery. According to ivGTT insulin sensitivity index (SI), pGDM were divided into insulin resistant (IR; SI<2.8 10^{-4} $min^{-1}/(\mu U/ml)$) or insulin sensitive (IS). The fatty liver index (FLI>60 =likelihood >78% presence of FL; FLI<20=likelihood >91% absence of FL) was calculated for each group and was correlated with metabolic parameters.

Results: Women with pGDM presented significantly higher FLI levels than women with NGT (30.0±3.6[SEM] vs. 20.6±5.6[SEM], p=0,008). In particular IR women showed higher FLI-values compared to IS (45.9±6.5 vs. 19.2±3.3, p=0.002). 16.1% of pGDM vs. 10.7% of NGT had FLI-values >60. In the whole sample FLI was negatively correlated with OGIS (r_S=-0.58, p<0.001) and HDL-cholesterol (r_S =-0.45, p<0.001). In addition, FLI was positively correlated with systolic and diastolic blood pressure, CRP, usCRP and liver enzymes. Further, we found a negative association with Adiponectin as well as a positive association with Leptin.
Conclusion: Results suggest that women with pGDM are at higher risk for fatty liver disease. In particular insulin resistance is associated with significantly high FLI-values indicating that fatty liver could be another early marker for progression of the disease.

Parameters included in FLI

	PGDM	NGT	p-value
BMI (kg/m^2)	27.4±0.7	25.4±1.2	0.113
Triglycerides (mg/dl)	111.7±7.9	90.1±8.6	0.102
γGT (U/l)	8.6±0.6	11.5±2.0	0.083
Waist (cm)	92.4±1.8	80.8±3.1	0.001

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Changes in humoral autoimmunity in pregnant women with abnormal glucose tolerance

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Background and aims: Autoantibodies (AAs) against pancreatic β-cell antigens cause autoimmune destruction, followed by β-cell dysfunction. The presence of autoimmune markers in pregnant women is the potential for increased risk for the future development of type 1 diabetes mellitus (T1DM). The aim of the study is to evaluate the changes in hummoral autoimmunity in high risk for T1DM pregnant women with normal and abnormal glucose tolerance.
Materials and methods: A one year prospective study among 96 pregnant women was performed in the period March 2008 - March 2009. A 75 grams Oral Glucose Tolerance Test (OGTT) was performed between 24 - 26 gestational weeks. The levels of blood glucose (BG) and immunoreactive insulin (IRI) has been measured at 0 min., 60 min. and 120 min. According to the results of OGTT the patients were divided into two groups: pregnant with normal carbohydrate tolerance (g_1= 53) and pregnant with impaired carbohydrate tolerance (g_2 = 43). The presence of antibodies against insulin (AIA), glutamic acid decarboxylase (GAD65) and one of the heat stress shock protein (AHspA) was examined in sera using indirect ELISA (AIA and AHspA) and EIA (GAD65) methods. All statistical analyses were performed with statistic panel - SPSS for Windows version 11.0.1. The difference between groups was compared by two tailed Student's *t*-test.
Results: Seven sera from the g_1 (13.2%) and five sera from the g_2 (11.6%) were positive for AIA (P>0.05). GAD65 autoantibodies were found in one serum from the g_1 (1.8%) and seven sera from the g_2 (16.2%); (P<0.001). Sequent analysis for AHspA has found two positive sera in g_1 (3.8%) and four positive sera in g_2 (9.3%); (P>0.05). There were statistically significant difference in total percentage of presence of autoantibodies between g_1 and g_2 groups (18.8% vs. 37.2%; P<0.03). We have fond positive correlation between BG levels during OGTT and presence of AHspA in g_2 (P<0.04). There was positive correlation between basal IRI levels at 0 min. and presence of AIA (P<0.02) in group$_2$. Four pregnant women positive for IAI of g_1 (51.7%) and three pregnant positive for IAI of g_2 (60.0%) have first and second degree relatives with T2DM. Five pregnant of GAD65 positive of g_2 (71.4%) have first and second degree relatives with T1DM.
Conclusion: These data support that autoimmune markers for pancreatic β-cell destruction are elevated in pregnant women with abnormal OGTT. In the absence of effective methods for prevention of T1DM measurement of immunologic markers in pregnant with high hereditary risk should be important tool for studying the progress of β-cell dysfunction.

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Pancreatic islets in human gestational diabetes

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Background and aims: Autoptic studies have shown an increased amount of islets and beta-cells in human pregnancy. However, no information is currently available on the properties of islet cells in human gestational diabetes. Here we describe several morphological, ultrastructural and functional properties of islets from a 33 yrs old woman with gestational diabetes (GD), who died for cerebral hemorrhage (rupture of a congenital aneurysm) at the 27th week of gestation. GD had been diagnosed at the 22th week, and therapy at time of death was with 32 IU insulin per day. At last control, fasting plasma glucose was 104 mg/dl and HbA1c was 6.1%.
Materials and methods: Immunohistochemical stainings and electron microscopy (EM) analyses were performed on pancreatic samples before islet isolation. Islet preparation was accomplished by collagenase digestion and gradient purification. Insulin secretion from the isolated islets was studied in response to glucose (3.3 and 16.7 mM), arginine (20 mM) and glibenclamide (100 μM).
Results: Islets area (diameter > 50 μm) was reduced in GD (0.55%) compared to non-diabetic controls (Ctrl) (1.32 and 1.47%), whilst islet insulin-positive area was higher in GD (66.0%) compared to Ctrl (51.9 and 47.2%). Apoptosis (cleaved caspase 3 immunohistochemistry and EM) was not different between the three cases, and Ki67 immunohistochemistry did not show replicating cells in the islets studied. The percentage of ducts with insulin-positive cells in the wall or within five nuclei far from it was not different between GD (27%) and Ctrl (45 and 23%); however, clusters (<10 cells) of insulin-positive cells scattered in the acinar tissue were more frequent in GD (1.35 per mm^2) compared to Ctrl (0.18 and 0.26 per mm^2). Ex-vivo insulin secretion was similar in GD and control islets.
Conclusion: In this case of human GD, a reduced amount of islets was observed; since apoptotic phenomena were similar in GD and control beta-cells, it is possible that not sufficient regeneration played a major role.
Supported by: European Community

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Hepatic and nonhepatic glucose uptake in a diet-induced model of gestational diabetes

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Background and aims: Glucose delivery via the hepatic portal vein (or the "portal signal") enhances net hepatic glucose uptake (NHGU) but reciprocally inhibits nonhepatic and skeletal muscle glucose uptake. Thus the portal signal directs the partitioning of a glucose load among the tissues but does not increase whole body glucose uptake. Activation of glucokinase (GK) might explain the liver's response. We have shown that feeding a high-fat and -sugar diet (HFD; energy: 60% fat, 12% fructose or sucrose) to pregnant (P) dogs during gestational wks 5-8 (total gest=9 wk) alters the response to an OGTT, resulting in a canine model of gestational diabetes (GDM). It also worsens P-induced whole body and muscle insulin resistance and impairs suppression of hepatic glucose output during a hyperinsulinemic euglycemic clamp. We hypothesized that glucose disposal under hyperinsulinemic, hyperglycemic conditions in the presence of the portal signal would be impaired in HFD-fed P dogs.
Materials and methods: P dogs with chronic vascular catheterization to allow assessment of hepatic and hindlimb balance received a meat/chow diet (C; 26% fat, 42% CHO [2% sugar]) or the HFD during gest wks 5-8; *n*=7/group. In overnight-fasted conscious dogs during the 8th gest wk, somatostatin was infused to disable the endocrine pancreas, and glucagon (basal), insulin (4×basal), and glucose (4 mg·kg^{-1}·min^{-1}) were infused via the portal vein for 4h. Glucose was infused i.v. as needed to clamp the hepatic glucose load at 2×basal.
Results: Clamp period arterial (157±1 and 158±1 mg/dl) and portal (172±2 mg/dl in both) blood glucose, and arterial plasma insulin (25±2 and 28±3 μU/ml) and glucagon (48±5 and 37±2 ng/l) were not different in C and HFD, respectively. Subsequent data are for the last h of the clamp unless otherwise stated, and data are for C and HFD groups, respectively. The total glucose infusion rate was not different between groups (9.6±0.6 and 9.4±1.0 mg·kg

body wt^{-1}·min^{-1}). NHGU was reduced in HFD (15.0±1.5 vs 5.8±3.0 mg·100g liver^{-1}·min^{-1},*P*<0.05), and net hepatic lactate uptake was enhanced (13.3±10.0 vs 42.3±7.6 µmol·100g liver^{-1}·min^{-1}, *P*<0.05). Net hepatic carbon retention (17.9±1.4 and 9.7±2.6 mg glucose equivalents·100g liver^{-1}·min^{-1}, *P*<0.05) was reduced and terminal hepatic glycogen concentrations tended to be reduced (3.7±0.8 vs 2.4±0.4 g/100g liver; *P*=0.17) in HFD. Hepatic GK activity did not differ between C and HFD. Hindlimb (an index of skeletal muscle) glucose uptake was higher in HFD (9.8±1.1 and 19.8±1.9 mg/min, *P*<0.05), and nonhepatic glucose uptake tended to be higher in HFD (5.9±0.7 and 8.0±1.2 mg·kg body wt^{-1}·min^{-1}, *P*=0.06).
Conclusion: NHGU in the presence of hyperinsulinemia, hyperglycemia and the portal signal was reduced in HFD-fed dogs, but not in association with impaired catalytic activity of GK. Despite the presence of insulin resistance in the HFD dogs, the reciprocal nature of liver and skeletal muscle glucose disposal was maintained such that there was no reduction in whole body glucose disposal. In P women with impaired glucose tolerance or GDM, blunted NHGU could contribute to postprandial hyperglycemia, increasing the peripheral glucose load.
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Maternal diabetes increases apoptosis in mice oocytes but not in 2-cell embryos
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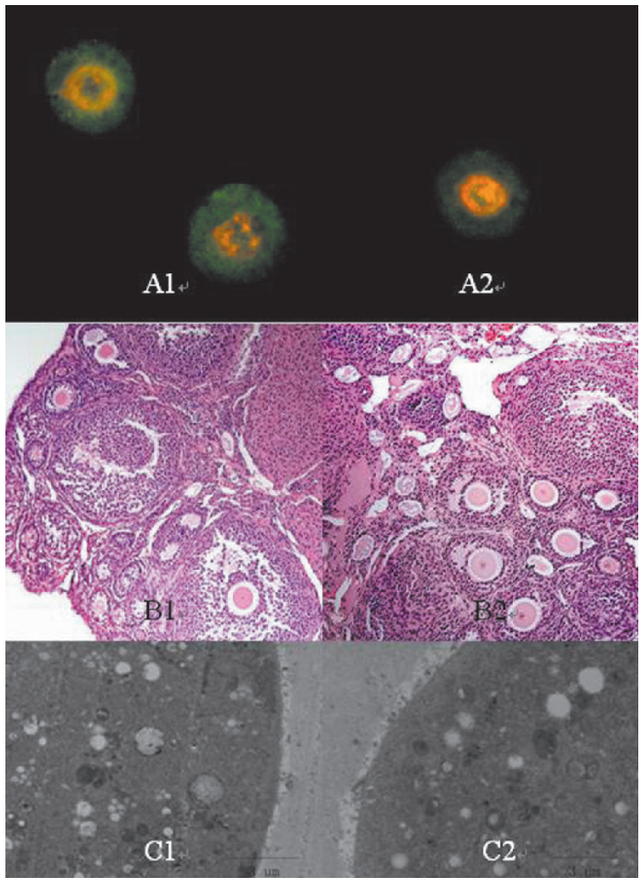
Background and aims: It has been reported that diabetes-induced inappropriate apoptosis in the developing embryos and oocytes may be one of the mechanisms leading to congenital malformation or miscarriage. The objective of this study was to investigate the occurrence of apoptosis at an early stage of development, the oocytes and 2-cell embryos in streptozotocin (STZ)-induced diabetic mouse.
Materials and methods: Bax and Caspase-3 protein and mRNA were detected respectively by Immunofluorescent and quantitative reverse transcription-polymerase chain reaction in oocytes and 2-cell embryos from diabetic vs nondiabetic mice. Apoptosis was detected by Annexin-V staining. Furthermore, HE-stained ovarian sections were made to see the effect of hyperglycemia on the oocytes maturation and development, electron microscopy was used to see the effect of hyperglycemia on the ultrastructure of 2-cell embryos.
Results: The increased number of Annexin-V-positive cells occurred in diabetic oocytes compared to nondiabetic oocytes (P<0.05). In quantitative RT-PCR and immunofluorescent, Bax and caspase-3 expression were significantly increased in diabetic oocytes than in nondiabetic oocytes (P<0.05). HE-stained ovarian sections demonstrated that hyperglycemia resulted in delayed follicular growth as detailed by reduced number of growing follicles (P<0.05) and a reduction in follicle size (P<0.01). In contrast, no any Annexin-V-positive cells in 2-cell embryos were found in diabetic and nondiabetic mice. Although Bax expression was elevated in diabetic 2-cell embryos (P<0.05), caspase-3 expression in 2-cell embryos was no significant difference between diabetic and nondiabetic mice (P>0.05). Electron-Microscopy study revealed that more swollen mitochondrias were found in diabetic 2-cell embryos.
Conclusion: Maternal diabetes might increase oocyte apoptosis by a Bax-caspase-3 pathway to play a role in embryonic malformations by delayed oocyte development. Development of 2-cell embryos might be adversely affected by maternal diabetes, but not through Bax-regulated caspase-3 apoptotic pathway.



Typical images were from diabetic (left) and nondiabetic (right) mice. Immunofluorescent showed Bax expression was higher in diabetic oocytes (A1) than nondiabetic oocytes (A2). HE-stained ovarian sections demonstrated mature follicles were less in diabetic ovary (B1) than nondiabetic ovary (B2). Electron microscopy revealed the proportion of swollen mitochondria was significantly higher in diabetic (C1) than nondiabetic 2-cell embryos (C2).

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Placental antioxidant capacity in gestational diabetes
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Increasing evidence in experimental and clinical studies suggests that there is a close link between hyperglycemia, oxidative stress and diabetic complications. Gestational diabetes mellitus (GD) is a pathological state of carbohydrate intolerance first recognized during pregnancy. The incidence of major congenital malformations is much higher in pregnancies complicated by diabetes and it has been suggested that oxygen free radicals are involved in the fetal dysmorphogenesis associated with diabetic pregnancies. Decreasing activities of antioxidant enzyme capacity could be part of the pathogenesis and thus the purpose of our study was to examine whether the level of the antioxidant biomarker superoxide dismutase (SOD) activity in placental tissue is altered in GDM. We studied 6 women with GD (GDM) (29,2±2,2 y.o.) and 7 age matched (31,6±1,7y.o.) (p=0,392) women with normal glucose tolerance (NGT) who served as controls. We measured a biomarker activity of antioxidant defense, namely superoxide dismutase (SOD) (colorimetric based assay) in placental tissue samples, taken while the surgery for caesarean section. Statistical analysis performed with unpaired T-test. All pregnancies were fully termed, uncomplicated with normal newborns with no difference in birth weight (GDM: 3209,17±181,3 vs NGT: 2956,43±65,5 g) (p=0,236). GDM group demonstrated significantly higher plasma insulin levels vs NGT (12,87±1,5 vs 6,70±0,8 µIU/ml) (p=0,008), while at euglycemic levels 82,83±7,5 vs 67,75±5,2 mg/dl (p=0,18) indicating lower insulin sensitivity in GDM. GHbA1c levels in GDM were within normal limits (5,10±0,6).

There was no difference in BMI between groups, before pregnancy (GDM: 24,76±1,3 vs NGT: 25,71±1,6) (p=0,66) as well as for the weight gained during pregnancy (GDM:13,58±1,6 vs NGT: 12,34±1,8 kg) (p=0,615). The placental superoxide dismutase levels were significantly lower in GDM than NGT (519,98±29,5 vs 671,17±49,1 μM) (p=0,028). In conclusion, the difference in the antioxidant biomarker level indicate that regardless the euglycemic condition in GD, there is an increased oxidative load and thus an exhaustion of the cell antioxidant capacity. These data may point toward a cellular pathway that may contribute in the higher prevalence of adverse outcomes in GD.

PS 107 Neuropathy - diagnostic tools

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Validity of DN4 as a screening tool for neuropathic pain of painful diabetic polyneuropathy

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Background and aims: Among the screening tools developed for distinguishing neuropathic from nociceptive pain, the DN4 (Douleur Neuropathique en 4 Questions) use both interview questions (DN4-interview) and limited bedside physical tests. DN4 has not been specifically validated in diabetes. This study was aimed at assessing the validity and diagnostic accuracy of DN4 as a screening tool in identifying neuropathic pain associated with painful diabetic polyneuropathy (PDPN).
Materials and methods: In 135 diabetic patients (age 53±16 years, diabetes duration 16±12 years, BMI 30±7 Kg/m^2, 68 male, 38 with type 1 diabetes), clinical parameters, symptoms and signs of diabetic polyneuropathy (DPN), the vibration perception threshold (VPT), the cold (CTT) and warm sensation thresholds (WTT), and neuropathic pain were evaluated using the Michigan Neuropathy Screening Instrument Questionnaire (MNSI-Q), the Michigan Diabetic Neuropathy Score (MDNS), the Biothesiometer, the Neuro Sensory Analyzer TSA-II, a pain history, an 11-point numerical rating scale (NRS), the Short-Form McGill Pain Questionnaire (SF-MPQ), the Brief Pain Inventory (BPI), and DN4 (the latter administered by "a blinded" investigator). PDPN was defined as the presence of DPN (at least 2 abnormalities among symptoms, signs, VPT, thermal thresholds, and 10 g monofilament) plus clinician-diagnosed chronic neuropathic pain with the same distribution as the neurological deficits and attributable to DPN.
Results: Forty-five patients fulfilled the criteria for PDPN (PDPN$^+$), 33 for DPN (DPN$^+$) and 57 were DPN free (DPN$^-$). The DN4 score was related to both neurological (Vs. MNSI-Q: rho=0.82, P<0.0001; Vs. MDNS: rho=0.62, P<0.0001; Vs. VPT hallux: rho=0.48, P<0.0001; Vs. number of 10 g monofilament correct answers: rho=-0.42, P<0.0001; Vs. CTT: rho=-0.28, P=0.0026; Vs. WTT: rho=0.31, P=0.0009) and neuropathic pain indices (Vs. 24h average pain NRS: rho=0.30, P=0.049; Vs. SF-MPQ sensory dimension: rho=0.56, P=0.0003; Vs. BPI pain now: rho=0.43, P=0.005; Vs. BPI general activity interference: rho=0.33, P=0.029; Vs. BPI mood interference: rho=0.32, P=0.033). Using ROC analysis, the DN4 score showed a high diagnostic accuracy in discriminating between PDPN$^+$ and both DPN$^+$ and DPN$^-$ (AUC: 0.95±0.02, 95% C.I. 0.91-0.98), as well as between PDPN$^+$ and DPN$^+$ or DPN$^-$ (AUC: 0.93±0.03 and 0.96±0.02, respectively). The DN4-interview score showed a similar diagnostic accuracy (AUC: 0.94±0.02, 95% C.I. 0.89-0.97). At the cut-off score of 4, DN4 displayed a sensitivity of 79%, a specificity of 92%, a positive predictive value of 84%, a negative predictive value of 89%, and a likelihood ratio for a positive result of 9.89. At the cut-off score of 3, DN4-interview displayed a sensitivity of 79%, a specificity of 85%, a positive predictive value of 74%, a negative predictive value of 88%, and a likelihood ratio for a positive result of 5.33.
Conclusion: This study demonstrates for the first time the diagnostic accuracy of DN4 in identifying neuropathic pain in PDPN and supports its usefulness as a screening tool for diabetic peripheral neuropathic pain. The DN4-interview has quite similar diagnostic accuracy as the full version of DN4 with the additional advantage of a possible use as a self-questionnaire.

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Neuropad and corneal confocal microscopy: new indicators for human diabetic neuropathy

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Objectives: Evaluation of early nerve damage is important to define diabetic patients at risk of developing pain and foot ulceration. The earliest nerve damage occurs to the small fibres detected by skin biopsy. Corneal confocal microscopy (CCM) and the Neuropad test provide non-invasive structural (c-fibres) and functional (sympathetic cholinergic fibres) measures of small fibre damage and may act as screening tools for diabetic neuropathy.
Methodology: 100 diabetic patients (Type I/II: 61/39) and 14 healthy volunteers underwent assessment of neuropathic severity using the Neuropathy

disability score (NDS), vibration perception threshold (VPT), Neuropad assessment, and CCM.
Findings: All control subjects had a normal response to Neuropad (100% Pink). Patient response was classified into three groups: Normal response (100% Pink), Intermediate response (patchy pink), and abnormal response (100% Blue).

Demographic characteristics of the controls and patients according to the three categories of Neuropad responses.

	Controls	Diabetic Patients		
	Normal response	Normal response	Patchy response	Abnormal response
Number	14	31	43	26
Age (yrs)	48 ± 2.50	46 ± 3	53.5± 2	52± 2.50
Type diabetes (I/II)	-	19/12	22/21	20/6
Duration of diabetes (yrs)	-	15± 2.00	22.00± 2.00	21± 2.00
HbA1c (%)	<6.5	7.5 ± 0.3	7.9± 0.3	8.0 ± 0.5
NDS (0-10)	0	1.3±0.3	4.5±0.5	6.3±0.7
VPT(Hz)	6.5±0.4	10.5±1.1	19.4±1.8	28.5±3.1#
NFD (no/mm^2)	48.3±3.3	32.9±1.8#	25.0±2.1*	16.5±2.5*
NBD (no/mm^2)	30.1±1.4	17.0±2.1*	11.4±1.4*	7.8±1.7*
NFL (mm/mm^2)	9.7±0.7	6.0±0.6	4.3±0.4	3.0±0.6*

Post Hoc: v control *P<0.001, #P<0.05,

Diabetic patients with a normal NDS and VPT i.e. no neuropathy have a normal Neuropad response which becomes patchy in those with mild neuropathy and abnormal in those with more severe neuropathy. However, CCM abnormalities are present even in patients with no neuropathy and progressively worsen with increasing neuropathic severity. The Neuropad response correlated with NDS (rs=0.456, p= 0.000), VPT (rs= 0.330, p=0.000), NFD (rs=-0.365, p=0.000), NBD (rs=-0.377, p=0.000), and NFL (rs=-0.395, p=0.000).
Conclusion: Neuropad therefore detects mild neuropathy whilst CCM detects nerve damage at the very earliest stage. As both tests are non-invasive they offer considerable potential as screening tools.

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A simple method for detecting 'at risk feet' in hospitalised individuals with diabetes

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Background and aims: Approximately 3% of hospitalised individuals with diabetes develop a foot lesion during admission. Yet, care providers infrequently screen patients on admission for at-risk feet citing the unavailability of screening instruments. We wanted to assess a novel, simple, readily-available method - the touch test [TT] - to detect sensory neuropathy.
Materials and methods: The TT involves lightly touching with the operator's finger the dorsum of 1st and tips of 1st, 3rd, 5th toes bilaterally. We assessed the performance of monofilament [MF] and TT in 122 diabetic subjects against a vibration perception threshold of >25V from neurothesiometry in either foot. Assessments were performed by 8 individuals.
Results: MF and TT have similar and good sensitivity, specificity and operating characteristics. Applying the criterion of ≥2/8 insensate areas to signify neuropathy, the sensitivities and specificities for TT were 70% and 88% (MF 78% and 91%), The area under the curve for TT is 0.80 (MF, 0.85). Inter-rater agreement for TT indicated substantial agreement, Cohen's kappa (k) = 0.68.
Conclusion: This equipment-free test which is 'an arms length away' performs well against a recognised standard known to predict risk of ulceration. Health care providers should use this simple, handy test to assess all patients admitted with diabetes.

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Comparing skin biopsy with corneal confocal microscopy: diagnostic yield of nerve fiber density

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Background and aims: The assessment of intra-epidermal nerve fiber density (IENFD) in skin biopsies and corneal nerve fiber density (CNFD) using corneal confocal microscopy (CCM) provides promising techniques to detect small nerve fiber damage in patients with peripheral neuropathy. To help define the clinical utility of each of these techniques in patients with diabetic neuropathy we have assessed sensitivity and specificity of IENFD and CNFD in predicting the following: 1) diabetic polyneuropathy (DPN); 2) risk of foot ulceration (RFU); 3) initial small fiber neuropathy (iSFN); 4) severe small fiber neuropathy (sSFN).
Materials and methods: 55 diabetic patients underwent assessment of neuropathic deficits using neuropathy disability score (NDS), nerve conduction studies, vibration perception threshold (VPT) using the Neurothesiometer, cooling detection threshold (CDT), minimal heat-as-pain threshold (0.5 HP-VAS) and autonomic function testing (DB-HRV) using the CASE IV. Definition of DPN was: 1) NDS ≥ 3/10, 2) peroneal motor nerve conduction velocity (PMNCV) ≤ 42 m/sec, 3) at least one positive small fiber assessment among DB-HRV≤5th pc, CDT≥95th pc, HP-VAS≥95th pc (CASE IV normality range); 4) VPT ≥ 15 V. Definition of RFU was: 1) NDS ≥6/10, 2) VPT ≥25, and one among the following: PMNCV ≤35 m/sec, CDT ≥98th pc, HP-VAS ≥98th pc, DB-HRV ≤3rd pc. Definition of iSFN was: any value outside the 5th - 95th pc range (whichever end of the distribution applied) for CDT, 0.5 HPVAS and DB-HRV Definition of sSFN was: all values outside the same range for all available CDT, 0.5 HPVAS and DB-HRV. All patients then underwent skin biopsy to quantify IENFD and CCM to quantify CNFD.
Results: The sensitivity of IENFD in diagnosing DPN was 78%, RFU-88%, iSFN-69% and sSFN-86% with respective specificities of 56%, 54%, 63% and 46%. The sensitivity of CNFD in diagnosing DPN was 56%, RFU-63%, iSFN-50% and sSFN-86% with respective specificities of 75%, 72%, 84% and 69%.
Conclusion: Although the sensitivity for detecting different severities of DPN is greater for IENFD compared to CNFD, the specificities for the latter are greater. Specifically for detecting SFN, CNFD has a comparable and high sensitivity but higher specificity than IENFD. However, the major advantage of CCM is that it is a non-invasive technique.

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Extensor digitorum brevis muscle atrophy in diabetic patients

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Background and aims: Diabetic polyneuropathy is clinically sensory dominant. However, electrophysiological assessment *i.e.* F-wave latency study indicates early motor fibre involvement even in an asymptomatic patient. We assessed atrophy of extensor digitorum brevis (EDB) muscle in diabetic patients, whether this small foot muscle atrophy has diagnostic value for diabetic polyneuropathy or not.
Materials and methods: We examined EDB muscle atrophy and other neuropathic signs in 42 diabetic patients, and score of the michigan neuropathy diagnostic instruments (MNDI) was assessed. Motor and sensory nerve conduction studies were carried out in the tibial, peroneal and sural nerves bilaterally. The EDB muscle atrophy was graded as follows: Grade 0; no atrophy, Grade I: just wasted and visible, Grade II; palpable or visible by toes dorsiflexon, Grade III; neither palpable nor visible.
Results: EDB muscle atrophy was clearly and easily detectable by inspection and palpation. Number of patients of each group was as follows: G-0; 7, G-I; 15, G-II; 13, G-III; 7. Average scores of MNDI are: G-0; 2.4, G-I; 3.5, G-II; 3.7, G-III; 3.9. CMAP amplitudes of EDB after giving supramaximal electric shock to the deep peroneal nerve at the ankle are: G-I; 2.9mV, G-II; 1.4mV, G-II; 0.9mV, G-III; 0.3mV. Distal latency time and MCV were most abnormal in G-III. CMAP of the abductor hallucis muscle evoked by the tibial nerve stimulation and CSAP of the sural nerve were as follows, respectively: G-0; 10.5mV, 6.2uV, G-I; 8.0mV, 5.8uV, G-II; 7.9mV, 6.0uV, G-III; 2.9mV, 2.3uV.

Distal latency, MCV and Minimal F-wave latency of the tibial nerve, and SCV of the sural nerve were markedly delayed in G-III patients.
Conclusion: EDB muscle atrophy well reflects clinical and electrophysiological severity of diabetic polyneuropathy, and appears to be a simple and useful sign indicating progressive motor fibre loss in diabetic polyneuropathy.

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Spinal cord atrophy and thalamic neuronal dysfunction in distal symmetrical diabetic polyneuropathy

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Central nervous system involvement in diabetic sensorimotor neuropathy (DN) is being increasingly recognised. We have previously demonstrated the presence of spinal cord atrophy not only in subjects with established-DN but also in early, subclinical DSP. In a further proton magnetic resonance spectroscopy (H^+MRS) study, subjects with established-DN were found to exhibit thalamic neuronal dysfunction. In this study we looked at the relationship between spinal cord atrophy and thalamic neuronal function in DSP.
Methods: Twenty subjects [6 healthy volunteers; 14 diabetic subjects (8 No-DN and 6 Established-DN)] underwent detailed assessment of peripheral nerve function including: neuropathic symptom evaluation, clinical examination to determine the Neuropathy Impairment Score of Lower Limbs (NIS[LL]), 5 attributes of nerve electrophysiology, vibration detection threshold and heart rate with deep breathing test. MR imaging of the cervical spine and thalamic H-MRS were conducted within one year. Cervical cord cross-sectional area at disc level C2/C3 was calculated. Established markers of neuronal function [N-acetyl aspartate:choline (NA:Cho)] were obtained from the thalamus using long echo time H^+MRS.
Results: There was no significant difference in subgroup demographics [age: HV 42.5(15.4), No-DN 42.8(8.7), and Established-DN 48.4 (11.3)]. NIS[LL]+7 test score was significantly higher in subjects with Established-DN [21.8(15.2)] compared with No-DN [1.14(1.07); $p<0.05$]. Subjects with Established-DN had significant reduction in both cervical cord area [ANOVA p=0.002; Established-DN mean(SD) 52.1(4.1) vs No-DSP 68.8(8.20), p=0.001 and Established-DN vs HV 68.7(10.4), p=0.002] and thalamic NA:Cho [ANOVA p=0.013; Established-DN 1.58(0.16) vs No-DN 1.80(0.15) and Established-DN vs HV 1.74(1.0)] compared to the other groups. Among subjects with diabetes, cervicalcord area was significantly related to thalamic NA:Cho (r=0.42, p=0.04).
Conclusion: Diabetic subjects with Established-DN had the greatest level of spinal cord atrophy and thalamic neuronal dysfunction compared those with No-DN and HV. Significant positive correlations between spinal cord area and thalamic NA:Cho suggest progressive, concomitant involvement of the central nervous system in DN. The pathophysiological insult on the nervous system caused by DN appears more generalised, involving both the peripheral and central nervous systems.
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Symptomatic neuropathy in type 1 diabetes is preceded by subclinical electrophysiological abnormalities - a prospective study

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Background and aims: It is assumed that overt neuropathy with symptoms is preceded by a subclinical form which can be detected by studies of nerve conduction velocity. However, long-term studies in patients with type 1 diabetes with multiple insulin-injection therapy are lacking. The aim of the study was to elucidate if subclinical electrophysiological abnormalities can predict symptomatic neuropathy in patients with type 1 diabetes.
Materials and methods: Fifty-nine patients with type 1 diabetes were examined twice, 43 already in year 1994, and 16 in year 2000. From onset of diabetes all patients were treated with multiple insulin-injection therapy. Duration of diabetes at the second examination was 20.1 +/- 5.4 (range 10-31) years. The first examination included motor nerve conduction velocity (MCV), compound muscle action potential (CMAP) in peroneal and median nerves and sensory nerve conduction velocity (SCV) and nerve action potential (SNAP) in sural and median nerves. The second examination included also assessment of quantitative sensory thresholds (QST), neuropathy impairment assessment (NIA) and neuropathy symptom assessment (NSA). Symptomatic neuropathy was defined as NSA of ≥ 1.
Results: At the second examination symptomatic neuropathy was found in 12/ 59 (20%) of the patients. Those with symptoms on follow-up had lower peroneal and median MCV at baseline compared to those without symptoms ($p < 0.05$ for both parameters). In a logistic regression model, baseline long-term HbA1c and baseline median MCV predicted symptoms of neuropathy at follow-up. A decrease in peroneal MCV by more than two standard deviations at baseline significantly increased the risk of symptomatic neuropathy (OR=17; $p < 0.03$) A combination of both long term HbA1c ≥ 7.0 and a baseline decrease in peroneal MCV (≥ 2 SD) increase the risk of future symptoms (OR=21; $p<0.000$). In addition, the decrease in peroneal MCV at baseline showed a positive correlation with all parameters of nerve conduction at follow up ($p < 0.003$).
Conclusion: Despite multiple insulin-injection therapy symptomatic neuropathy is seen in 20 percent of patients after 20 years of type 1 diabetes. Overt diabetic neuropathy with symptoms is preceded by a subclinical form detectable by studies of nerve conduction.
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PS 108 Somatic neuropathy - clinical observations

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Significant impact of mood disturbances on pain perception in painful DPN: time to re-evaluate current practice?

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Painful diabetic peripheral neuropathy (DN) is common and has a negative impact on mood, functionality and quality of life. Unfortunately, current clinical practice focuses on pharmacological interventions directed at pain relief without adequate assessment of mood. An understanding of the impact of mood disorders on pain reporting and perception may result in better characterisation of the pain experience and patient tailored management strategies.
Method: 60 patients with painful DPN [mean age: 56.5(10.2), male:61%, type 2 DM: 79.7%] underwent: 1) clinical examination and nerve conduction studies to quantify DPN; 2) assessment of pain intensity with Neuropathic Pain Scale (NPS) and 3) assessment of mood with Hospital Anxiety and Depression Scale, Pain Acceptance Questionnaire (CPAQ) and Pain Catastrophising Scale (PCS). Based on these assessments on subjects that had been referred to our tertiary, specialist painful DN clinic were found to have severe symptoms [diabetes duration: 13.9(8.3), pain intensity VAS: 7.2(2.1)].
Results: All subjects had moderate to severe painful DPN with group average NIS(LL) + 7 test score of 26.9(14.1). There was a high prevalence of mood disturbance with 70% suffering with either anxiety and/or depression. The presence of anxiety appears to result in reporting a greater variety of pain [anxiety vs no anxiety, NPS: 5.3(1.8) vs 4.3(1.1); p=0.03] and significantly higher pain scores [NPS: 6.2(1.9) vs 4.6(2.2); p=0.002]. However, subjects who were depressed were less likely to accept pain [CPAQ depression vs no depression, pain willingness: 20.6(11.8) vs 40.0(14.9); p=0.009] and engage in social and/or physical activity [CPAQ, activities engagement: 29.0(11.8) vs 46.1(18.3); p=0.04]. Anxiety [PCS: 5.2(3.0) vs 2.0(1.5); p=0.03] and depression [PCS: 5.4(2.8) vs 1.9(1.7); p=0.01] both result in a significant magnification of symptoms.
Conclusion: This study found a very high prevalence of mood disorders in patients with severe painful DN. It also highlights the differing but significant impacts of anxiety and depression on an individual's pain experience. As we have quick and simple screening tools and effective treatments for mood disorders, an appreciation/assessment of the psychological impact of mood on pain may lead to better clinical outcomes for sufferers.

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Prevalence of painful diabetic neuropathy and quality of life in patients with type 1 or type 2 diabetes mellitus in specialist care

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Background and aims: Chronic painful distal neuropathy (CPDN) is a major complication in diabetes mellitus. However, little is known about prevalence, severity, determinants and impact of CPDN on quality of life. This information is necessary to improve care of patients as well as to design preventive strategies and evaluate current and future therapies. In this observational study we assessed prevalence and effect on quality of life in patients with CPDN in one large hospital in the Netherlands.
Materials and methods: A total of 720 patients with either type 1 (DM1, n=334)) or type 2 (DM2, n=386)) attending the out-patient clinic for Diabetology of the University Medical Centre Utrecht received a set of questionnaires by mail. Response rate in DM1 was 50.6% and in DM2 51.0%. Questionnaires sent were pain visual analogue scale (0-10 point VAS), a 0-100 rating scale of quality of life on the day of the questionnaire (S100) and 3 quality of life (QoL) questionnaires (Euro-QoL EQ-5D, QoL-Enjoyment and satisfaction, and Medical outcomes study sleep scale). Other study data were collected from medical records. A diagnosis of CPDN was established when vibration sense at the level of the hallux was diminished or absent in combination with a VAS-score ≥4. No obvious other causes of painful neuropathy (severe alcoholism, inflammatory nerve damage, untreated B12 deficiency) were present.
Results: Mean age DM1 44.1±12.9, DM2 61.9±12.6 years (p<0.001); duration of disease DM1: 25.9±14.7, DM2: 15.0±8.3 years (p<0.001); males: DM1: 43.5; DM2: 60.2% (p<0.01); 93.4% patients with DM2 on insulin. Prevalence of CPDN in DM1 was 13.4% and 26.6% in DM2 (p<0.001). Both in DM1 and DM2, CPDN had a major, statistically highly significant (p<0.001) negative effect on daily activities, mood and well-being, professional activities, domestic and family activities, social relations, sexual activities as well as on sleep quality. S100 was 57.6±19.3 with pain and 77.0±16.5 without pain in DM1 (p<0.001); percentages in DM2 50.9±18.8 and 70.2±18.8 (p<0.001). S100 was significantly higher in DM1 than in DM2 in subjects without CPDN whereas S100 was comparable between DM1 and DM2 with CPDN.
Conclusion: In conclusion, chronic painful distal neuropathy is a common feature in both type 1 and type 2 diabetes, significantly more so in DM2 compared to DM1 and has a major negative influence on all aspects of quality of life. Quality of life constitutes a major effect parameter.
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BMI and nerve dysfunction in diabetic patients

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Background and aims: Recent epidemiological studies have found a strong correlation between diabetic neuropathy and body weight. Furthermore small fiber neuropathy tends to develop within a few years of diabetes as relatively early complication. Early recognition of potentially modifiable risk factors for diabetic neuropathy - a known risk factor for foot ulcers - is crucial if we are to succeed in prevention of diabetic foot lesions. The aim of the present study was to investigate the relationship between BMI (modifiable risk factor) and small and overall nerve fiber dysfunction in diabetic patients.
Materials and methods: 278 consecutive diabetic patients (type 2) were investigated. Males=147, Mean age (yrs) was 63,31±11,25, Mean duration of diabetes (yrs) 12,85±8,56. The Neuropathy Disability Score (sensory signs) was used to identify those patients with overall nerve dysfunction (NDS≥3: abnormal). The sum of deficits of pain and cold sensation was used to identify any impairment of small fiber dysfunction (NDS_1≥2: abnormal). BMI was calculated as usual (kg/m^2). Statistical analysis was performed in a univariate and multivariate model (level of significance 0,05).
Results: 1) BMI<25 (NDS_1: 0,4±0,3), 25<BMI<29 (NDS_1: 0,53±1,05), BMI≥30 (NDS_1: 1,04±1,01). p<0,05 for every comparison between two groups. Correlation between BMI and NDS_1 was significant (p: 0,05 r^2:0,026). 2) BMI<25 (NDS: 0,7±0,8), 25<BMI<29 (NDS: 1,1±0,9), BMI≥30 (NDS: 2,01±2,0). p<0,05 for every comparison between two groups. Correlation between BMI and NDS was also significant (p: 0,05 r^2:0,026). 3) In the multivariate analysis BMI and duration of Diabetes were significant factors for overall nerve dysfunction (p<0,05).
Conclusion: The present study shows that nerve fiber dysfunction is also associated with the body weight e.g. elevated BMI. This finding supports the role of other factors (modifiable) apart from the already known in the pathogenesis and progression of diabetic neuropathy and prevention of foot ulcers. Larger studies are needed to confirm this finding.

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Association between peripheral nerve function and bone mineral density in patients with type 2 diabetes mellitus

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Background and aims: To investigate the association between peripheral nerve function and bone mineral density (BMD) in patients with type 2 diabetes mellitus(T2DM).
Materials and methods: A total of 169 patients with type 2 diabetes were enrolled. All the patients were tested with fasting blood glucose (FBG), postprandial 2h blood glucose (P2BG), hemoglobin A1c (HbA1c), triglycerides, cholesterol, urinary albumin excretion,serum , serum calcium, serum phosphorus, serum magnesium, serum bone alkaline phosphatase isoenzyme . Lumbar and hip BMD were measured by dual-energy X-ray absorptiometry and according to the T-score ,all the patients were categorized into the osteoporosis group (T-score≤-2.5), the osteopenia group(-2.5<T-score≤-1)and the control group(-1< T-score≤1). The factor affecting T-score was analyzed

too. Nerve function was assessed by 10-g monofilament detection, vibration threshold, and bilateral sural nerve amplitude(CMAP) and conduction velocit(NCV) y, peroneal amplitude and NCV on the dominant side, and median motor and sensory amplitudes and NCVs on the nondominant side.
Results: Among the female patients, a higher proportion of postmenopausal women in osteoporosis group compared with controls(P<0.05). Patients with osteoporosis had older age(P<0.05) and longer T2DM duration (P<0.05) than control group both in male and female patients .No difference of the parameters of laboratory was seen among the groups, while, except the parameter of bone metabolism, B-ALP was elevated in osteoporosis group compared with controls . monofilament detection, and peroneal NCV) and CMAP were significantly lower in Patients with osteoporosis as well as the vibration threshold was significantly higher in them compared with control group. Relative analysis showed that the lumbar spine and hip BMD of T2DM group had negative correlation with age, duration menopause, severity of diabetic neuropathy and positive correlation of B-ALP . After adjusting for age, diabetic course, B-ALP, menopause, poor nerve function (lower nerve conduction amplitude and velocity) was associated with lower T-score of both lumbar spine and hip BMD significantly.
Conclusion: Lower BMD was associated with poor peripheral nerve function (both sensory nerve and motor nerve) in the type 2 diabetes mellitus, diabetic neuropathy was an independent risk factors of osteoporosis inT2DM. The pathophysiological mechanisms of bone metabolism and diabetic neuropathy should be the concern of further research.

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High prevalence of vitamin D deficiency in type 2 diabetic patients with neuropathy

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Background and aims: The pro-inflammatory profile (IL-6, TNF-α, IL-1, IL-8) in monocytes from type 2 diabetic patients is down-regulated by 1,25(OH)2D3, and levels of acute-phase proteins are significantly associated with neuropathic deficits in diabetic patients. Several studies have demonstrated alterations in vitamin D metabolism in those patients and adverse outcomes associated with vitamin D insufficiency have been described in the human musculoskeletal, innate immune, and cardiovascular systems. We report a pilot study assessing the relationship between peripheral neuropathy in type 2 diabetic patients and vitamin D depletion.
Materials and methods: We conducted a prospective and observational study of 111 consecutive type 2 diabetic patients ambulatory treated in Diabetology Department between January and April 2009. Subjects were tested for deep tendon reflexes at patella, and Achilles, sensory loss using vibration perception with 128-Hz tuning fork by the on-off method, and pressure perception with 10 g Semmes-Weinstein monofilament. Exclusion criteria were neuropathy from other etiologies (e.g., familial, alcoholic, nutritional and uremic) and vitamin D supplementation. Following data were recorded: Age, body mass index (BMI), glycated haemoglobin (HbA1c), duration of diabetes, peripheral neuropathy status (PN), intact serum parathyroid hormone (PTH), serum 25-OH vitamin D (25OHD) concentration, total serum calcium concentration and serum creatinine.
Results: Overall, 111 patients were evaluated and the majority (55.85%) of them had peripheral neuropathy. Patients with PN were significantly older, 70.58±10.85 vs 57.57±12.27 years respectively (p<0.0001) and had significantly longer diabetes duration, 17.61± 9.52 vs 10.21±6.97 years respectively (p<0.0001). No significant differences were observed in BMI and HbA1c. Significantly decreased 25OHD levels were found in the PN group, 24.6±11.98 vs 34.74±17,26 nmol/l (p<0.0001). Frank vitamin D deficiency rate (25OHD < 50 nmol/l or 20 ng/ml) was significantly higher in PN group, 95% vs 79,5% (p<0,05). Vitamin D insufficiency rate (25OHD between 50-75 nmol/l or 20-30 ng/ml) did not differ significantly between the two groups 5% in the PN group vs 16.5%. PTH levels and creatininemia were significantly increased in the PN group (p<0.05).
Conclusion: In type 2 diabetic patients, peripheral neuropathy is associated with significantly lower levels of 25OHD. Although these data do not prove the existence of a causal link between vitamin D deficiency/insufficiency and PN, they have major clinical and therapeutic implications for the management of PN. Vitamin D-deficient status potentially increases risk of non vertebral and hip fracture in this older population with muscle weakness at higher risk for falls. Vitamin D deficiency/insufficiency also decreases antibacterial responses in this PN population at higher risk for foot ulcers. On the other hand, the importance of the Nerve Growth Factor, which is up-regulated by Vitamin D, has been established in the development of diabetic PN using diabetic animal model. Further studies are needed to understand the causes and to investigate the clinical significance of vitamin D replacement therapy in PN diabetic patients. Determining what is the adequate vitamin D level for PN diabetic patients and how much supplementation is necessary is now a matter of crucial concern in the management of diabetic PN.

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Association between symptoms of neuropathy, nerve conduction and levels of heat shock protein 27 in type 2 diabetes

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Background and aims: Increased levels of serum HSP27 (sHSP27) are associated with distal symmetric polyneuropathy (DSPN) in type 1 diabetic patients. However, the association between nerve function and sHSP27 has not been studied in subjects with type 2 diabetes (T2D) and impaired glucose tolerance (IGT). Thus, our objectives were to investigate the association between nerve conduction in the legs, symptoms of distal polyneuropathy and sHSP27 levels.
Methods: Subjects were consecutively recruited from the population-based Västerbotten Intervention Program; controls (n=39, m/f=19/20, mean age=61± 0.6 years), IGT (n=29, m/f=15/14, mean age=61± 0.8 years), T2D (n=51, m/f=30/21, mean age=61± 1.3 years). Nerve conduction studies were performed. Z-scores for motor conduction velocity (CV) of the peroneal nerve, and the sensory CV and amplitude of the sural nerve were measured and compiled into a composite Z-score of the right leg (Z-score leg). Neurological Disability Score (NDS), including examination of sensory perception, reflexes and muscle strength, were used to evaluate symptoms of neuropathy in the leg. NDS and Z-score leg were categorized into tertiles, respectively. sHSP27 levels were measured and divided into low and high levels.
Results: Subjects in the highest NDS tertile had lower sHSP27 levels (328 ± 287 pg/mL) compared to subjects in the lowest NDS tertile (558 ± 404 pg/mL, p=0.04). Subjects in the lowest tertile of Z-score leg were in the lowest sHSP27 group (63%) compared to the subjects in the highest group (38%, p=0.034). The highest tertile of Z-score leg was associated with high levels of sHSP27 (OR 3.8, 95% CI 1.2; 11.5, p=0.02); adjusted for age and sex. However, this association was attenuated when adjusted for T2D status (OR 3.1, 95% CI 0.9; 9.9, p=0.06).
Conclusion: In summary, increased sHSP27 levels were associated with an increasing Z-score of the leg; thus, a better nerve conduction, and fewer symptoms using the whole study population. The attenuation of the association when including diabetic status indicates an altered HSP27 production in T2D patients compared to controls and subjects with IGT.

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The relationship between brachial-ankle pulse wave velocity and peripheral neuropathy in type 2 diabetes

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Background and aims: Brachial-ankle pulse wave velocity (baPWV) has been shown to be a good surrogate marker of clinical atherosclerosis. The aim of the study was to determine the relationship between baPWV and peripheral neuropathy in patients with type 2 diabetes.
Materials and methods: We assessed 692 patients with type 2 diabetes (314 men, 376 women, mean age 56.9 ± 10.9 years, mean diabetes duration 7.9 ± 6.3 years). The intensity of neuropathic symptoms (pain, burning sensation, paresthesia, and numbness) was scored according to numeric visual analog scales. The total symptom score was calculated from the sum of each neuropathic symptom scores. The neurological assessment (ankle reflexes and 10-g monofilament test) was performed. The baPWV was measured using an automated device.
Results: In bivariate correlation analysis, the presence of peripheral neuropathy (increased total symptom scores or abnormal neurological assessment) was significantly correlated with maximal baPWV (r =0.127, p < 0.01), age (r =0.119, p < 0.01) and sex (r =0.128, p < 0.01). After analysis using independent t- test, the patients with peripheral neuropathy had higher maximal

baPWV, systolic blood pressure and subject number of female sex and older age compared with control (Table).
Conclusion: Peripheral neuropathy was significantly correlated with baPWV in patients with type 2 diabetes.

Comparison of anthropometric characteristics of diabetes with and without peripheral neuropathy

Variables	DPN (n = 253)	Control (n = 439)	*P*-value
Age (years)	59 ± 11	56 ± 11	< 0.01
Male (%)	63 ± 5	50 ± 5	< 0.01
Diabetes duration (years)	8.5 ± 7.1	7.6 ± 5.8	NS
Height (m)	1.6 ± 0.1	1.6 ± 0.1	NS
Weight (kg)	64 ± 10	64 ± 10	NS
Systolic BP (mmHg)	139 ± 21	135 ± 17	< 0.01
Diastolc BP (mmHg)	82 ± 12	81 ± 9	NS

PS 109 Neuropathy - experimental

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Dyslipidaemia and peripheral prediabetic neuropathy
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Background and aims: Evidence for the presence of diabetes-like neuropathy at prediabetic stage, prior to development of overt hyperglycemia, is emerging from both experimental and clinical studies. Until now, it has not been sorted out whether prediabetic neuropathy results from glucose intolerance, or other factors such as impaired insulin signaling, hypertriglyceridemia, hypercholesterolemia, and/or increased fatty acid concentrations come into play. This study was aimed at evaluating relative roles of the afore-mentioned factors in peripheral nerve function in prediabetic condition.
Materials and methods: Experiments were performed in Zucker lean and Zucker fa/fa rats, a model of prediabetes and obesity. Zucker fa/fa rats of 16 wks of age displayed obesity, glucose intolerance, hyperinsulinemia, hypercholesterolemia, and increased serum NEFA concentrations. They developed sensory nerve conduction velocity (SNCV) deficit and small sensory nerve fiber dysfunction manifest by thermal and mechanical hypoalgesia and tactile allodynia.
Results: In the Zucker fa/fa rats, a 4-wk treatment with the niacin derivative acipimox significantly reduced serum insulin ($p < 0.01$), NEFA ($p < 0.05$), and triglyceride concentrations ($p < 0.01$) without affecting impaired glucose tolerance and total and VLDL-LDL cholesterol concentrations. It also reversed SNCV deficit ($p < 0.01$) and alleviated small sensory fiber neuropathy.
Conclusion: Our findings suggest that impaired insulin signaling, hypertriglyceridemia, and/or increased fatty acids, but not impaired glucose tolerance or hypercholesterolemia are responsible for development of prediabetic neuropathy. The latter provides rationale for new early stage interventions, to stop progression of this devastating diabetic complication.

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Effects of long-term administration of moderate amounts of insulin on small and large peripheral nerve fiber function and structure in non-diabetic Wistar rats
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Background and aims: Insulin exerts neurotrophic and neuroprotective effects on peripheral nerves. In animal models of insulin deficient type 1 diabetes, trace amounts of insulin can ameliorate small and large peripheral nerve fiber dysfunction without influencing the level of glycemia. On the other hand, we have reported that chronic hyperinsulinemic hypoglycemia caused by insulinoma leads to peripheral nerve microangiopathy and large fiber degeneration in non-diabetic rats. Here we aim to investigate the effect of chronic treatment with moderate amounts of insulin on small and large peripheral nerve fiber function and structure in non-diabetic rats in the absence of severe hypoglycemia.
Mterials and methods: Sustained-release insulin pellets were subcutaneously implanted in 10-wk-old non-diabetic male Wistar rats (Ins-rats; n = 17) and delivered 2 to 4 IU/day of insulin continuously to these animals for 14 wks (14Ins-rats; n = 9) or for 30 wks (30Ins-rats; n = 8). Small and large peripheral nerve fiber function and structure of these animals were investigated using behaviorial, histologic and electrophysiological analysis. Age- and sex-matched untreated Wistar rats served as controls (14C-rats; n = 8 for 14 wks, 30C-rats; n=8 for 30 wks).
Results: Both 14Ins-rats and 30Ins-rats showed occasional mild to moderate reduction in blood glucose level of no less than 2.4 mmol/l during the observation period. Final HbA1c level was decreased by 13% ($p < 0.0005$) in 14Ins-rats and by 7% ($p < 0.005$) in 30Ins-rats compared with controls. Final body weight and serum insulin levels did not differ between 14Ins-rats and 14C-rats, whereas final body weight increased by 14% ($p < 0.005$) and serum insulin level increased by 104% ($p < 0.05$) in 30Ins-rats compared with 30C-rats. Behavioral analysis revealed unmyelinated nociceptive fiber dysfunction, as indicated by an 11 to 26% increase in tail flick latency to noxious heat stimulus ($p < 0.05$), until 14 wks after insulin treatment in 14Ins-rats compared with 14C-rats. We also found a 9 to 21% increase in tail flick la-

tency ($p < 0.05$) between 12 and 16 wks after insulin treatment in 30Ins-rats compared with 30C-rats. Final intraepidermal nerve fiber (unmyelinated) density was marginally decreased ($p = 0.057$) in 14Ins-rats compared with 14C-rats (mean ± SE; 47.7 ± 4.5/mm vs 60.0 ± 3.8/mm) and was unchanged in 30Ins-rats compared with 30C-rats (40.4 ± 6.7/mm vs 40.1 ± 4.7/mm). In contrast, final electrophysiological analysis of the sciatic-tibial nerve showed unchanged sensory (SNCV) and motor nerve conduction velocities (MNCV) in 14Ins-rats compared with 14C-rats, while SNCV increased by 10% ($p < 0.05$) and MNCV by 11% ($p < 0.01$) in 30Ins-rats compared with 30C-rats. The increased nerve conduction was associated with a 12% increase ($p < 0.05$) in myelinated fiber density in the tibial nerve in 30Ins-rats compared with 30C-rats.

Conclusion: Treatment of non-diabetic rats with moderate amounts of insulin, without causing severe hypoglycemia, appeared to induce transient unmyelinated (small) fiber dysfunction and loss for periods up to 16 wks, and led to increased myelinated (large) fiber function and density for the more extended period of 30 wks. These functional and structural changes in small and large peripheral nerve fibers merit further investigation to elucidate the mechanism whereby insulin exerts its effects on peripheral nerves.

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The comparison of peripheral nerve damage according to the glucose control period in the experimental diabetes

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Background and aims: Besides just tight glucose control, early intensive therapy has been reported to be more important for the prevention of diabetic micro-and macro-complication. However, it was not known exactly about the quantitative difference according to the timing delay in the glucose control and whether early period control is really better than late control in the diabetic peripheral neuropathy. Therefore, in this study we investigated the effect of timing difference in glucose control on the peripheral nerves in the course of diabetes.

Materials and methods: The five groups (6-8 number in each group) comprised : Normal glucose rats (Normal), rats with hyperglycemia (designated: DM), rats with glucose control for entire 28-week period (designated: INS (W0-28)), rats with glucose control for early 14-week period followed by hyperglycemia for late 14 weeks (designated: INS (W0-14)), and rats with hyperglycemia for early 14 weeks followed by glucose control for late 14-week period (designated: INS (W15-28)).

Results: In the results, the current perception threshold (CPT) was more reduced in INS (W0-28) and INS (W15-28) group compared with INS (W0-14) or DM group (*P<0.05*). Mean myelinated axon area was larger significantly in INS (W0-28) and INS (W15-28) group (63.5±2.32 and 60.1±2.14 um) than INS (W0-14) or DM group (55.5± 2.81 or 51.5± 2.64 um) (*P<0.05*) and intraepidermal nerve fibers (IENF) density was less reduced significantly INS (W0-28) and INS (W15-28) group (6.9±0.46 and 6.8±0.11) than INS (W0-14) or DM group (5.9.5± 0.32 or 5.3± 0.39) (*P<0.05*). More increased trend of nerve fiber quantity was also observed in INS (W0-28) group than INS (W15-28) group although there was no significant difference.

Conclusion: Our results indicate that continuous glucose control is necessarily important to alleviate the peripheral nerve damage and moreover poor glycemic control during the later period prone to aggravate the neuropathy is more harmful than inappropriate early period management. Therefore, besides earlier management, the importance of continuous glucose control including later period of diabetes should also be emphasized in the diabetic peripheral neuropathy.

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Amelioration of diabetic enteropathy in chronic experimental diabetic rats transplanted with autologous adipose-tissue-derived mesenchymal stem cells

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Background and aims: Gastroenteropathy is a serious complication of diabetes and impairs quality of life, leading to bleak prognosis in diabetic patients. There is no effective treatment for this serious disorder. Cell therapy using adipose-tissue derived mesenchymal stem cells (ADSC) is now a promising approach for the reparative therapy and we applied ADSC to enteropathy in chronic diabetic rats.

Materials and methods: Streptozotocin-induced diabetic rats with 16 wk-duration were transplanted with autologous ADSC ($x10^6$), retrieved from subcutaneous region prior to diabetes onset, into the serosa of terminal ileum. After 6 wk observation period, thickness of mucosal villi, mRNA expressions of various growth factors and neuronal nitric oxide synthase (nNOS) as well as choline acetyltransferase (CHAT), and neuropathology of intestinal walls were examined in transplanted diabetic rats and compared with those in untreated diabetic rats and non-diabetic control animals.

Results: Mucosal villi in the intestine were thickened in diabetic rats and ADSC transplantation (Tx) nearly normalized the thickness of the mocosal wall. Neuronal distribution as demonstrated by PGP9.5 staining disclosed significant reduction of myenteric neuronal area and intramural axonal fibers in diabetic rats and these changes were improved by ADSC-Tx. Consistent with PGP9.5 staining, tissue contents of PGP9.5 were reduced in diabetic rats and corrected by Tx. Similarly, expressions of both PKB/Akt and pAkt were suppressed in the intestinal tissues of diabetic rats and corrected by Tx. There were also reduced mRNA expressions of fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-I, nNOS and CHAT in the intestine of diabetic rats, and Tx all upregulated the expressions. There was no significant influence of Tx on the mRNA expressions of these factors in normal control rats. The microscopy of transplanted sites revealed increased microvessels and sparse infiltration of inflammatory cells in diabetic and normal control animals.

Conclusion: The results demonstrated that topical application of autologous ADSC into the gut wall improved diabetic enteropathy in rats, warranting the future clinical application of ADSC transplantation for hitherto untreatable condition of diabetic enteropathy.

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Oxidative stress damage at pain control serotoninergic and noradrenergic brainstem centres during diabetic neuropathy

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Background and aim: Painful diabetic neuropathy was recently shown to be due to central mechanisms, both in humans and animals. Loss of serotoninergic and noradrenergic neurons at brainstem pain control centres was shown to occur in streptozotocin-diabetic rats (STZ-rats). This impairment in descending modulation may account for spinal hyperactivity and exacerbated pain behavioural responses. Oxidative stress is common in diabetes, affecting peripheral sensory system and central areas involved in cognition and memory. Here, we evaluated the occurrence of oxidative stress damage at serotoninergic and noradrenergic brainstem areas directly involved in descending pain modulation.

Materials and methods: Male Wistar rats were injected with streptozotocin (60 mg/kg) or saline and were sacrificed at 10 weeks post-injection. Brainstem sections were immunoreacted for thryptophan hydroxylase (TpH) and tyrosine hydroxylase (TH) to detect serotoninergic and noradrenergic neurons, respectively; and for 8-hydroxy-2'-deoxuguanosine (8-OH-dG), the marker of oxidative DNA damage. Rostroventrolateral medulla (RVM) was analysed for TpH immunoreactivity. The A5, A6 and A7 noradrenergic cell groups were studied for TH expression. Another set of sections was analysed for 8-OH-dG expression in the above mentioned serotoninergic and noradrenergic areas. Data were compared by independent sample *t* test and presented as mean±SEM.

Results: STZ-rats presented marked hyperglycaemia and behavioural signs of painful diabetic neuropathy (mechanical hyperalgesia and tactile allodynia evaluated by the Randall-Selitto and von Frey tests, respectively). Significantly lower numbers of TpH labelled neurons were detected at RVM of STZ-rats (STZ: 4.9±1.71; Saline: 15.8±4.17). Regarding the noradrenergic cell groups, significant low numbers of TH immunoreactive neurons were detected at the A5 (STZ: 9.8±0.73; Saline: 13.9±1.34) with a tendency for reduction in A7 cell group (STZ: 15.6±3.49; Saline: 21.6±2.17). No difference was detected at the A6 noradrenergic cell group. Oxidative stress damage was significantly higher in RVM (STZ: 11.2±2.60; Saline: 1.6±0.80), A5 (STZ: 18.0±5.22; Saline: 4.9±1.49) and A7 (STZ: 10.1±2.49; Saline: 2.3±0.59) noradrenergic cell groups in STZ-rats. In the A6 noradrenergic cell group, oxidative damage was similar in both groups.

Conclusion: The association between decreases in the numbers of serotoninergic and noradrenergic neurons and oxidative stress damage suggests that this may be a leading mechanism in the impairment of inhibitory descending pain modulation in STZ rats. It is likely that a reversal of oxidative stress damage at the brainstem should prevent the observed neuronal losses. This study shows the importance of increasing the intake of antioxidants in diabetic patients in order to control neuronal dysfunction and pain during diabetic neuropathy.
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Role for endoplasmic reticulum stress in prediabetic and diabetic neuropathy
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Background and aims: Endoplasmic reticulum (ER) stress caused by accumulation of unfolded proteins in the ER lumen, contributes to beta-cell loss, insulin resistance, and plays an important role in the pathogenesis of both Type 1 and Type 2 diabetes. Taking into consideration that ER stress is associated with impaired cell signaling and oxidative damage, we evaluated the role of this phenomenon in the pathogenesis of prediabetic and diabetic peripheral neuropathies.
Materials and methods: The experiments have been performed in 1) Zucker lean and Zucker *fa/fa* rats, a model of obesity and Type 2 prediabetes, and 2) control and streptozotocin (STZ)-diabetic rats, a model of Type 1 diabetes. Peripheral neuropathy endpoints included sciatic motor nerve conduction velocity (MNCV), hind-limb digital sensory nerve conduction velocity (SNCV), thermal algesia (paw withdrawal latency), tactile allodynia (tactile response thresholds), and intraepidermal nerve fiber density (fluorescent immunohistochemistry). ER was evaluated by expression of BiP/GRP78 and GRP94 in the sciatic nerve (Western blot analysis).
Results: Both 16-wk-old Zucker *fa/fa* rats and STZ-diabetic rats with 12-wk duration of diabetes displayed ER stress response manifest by overexpression of BiP/GRP78 and GRP94 in the peripheral nerve. They also had nerve conduction velocity slowing and small sensory nerve fiber dysfunction. STZ-diabetic rats displayed reduced intraepidermal nerve fiber density. Treatment with the chemical chaperone trimethylamine N-oxide (TMAO), 1 mmol $kg^{-1}d^{-1}$ in the drinking water, for 4 wks, reversed SNCV deficit and alleviated thermal hypoalgesia and tactile allodynia in Zucker *fa/fa* rats. The same treatment also prevented SNCV deficit and partially prevented MNCV deficit and small sensory nerve fiber dysfunction and degeneration in STZ-diabetic rats. In both studies, TMAO did not affect blood glucose concentrations.
Conclusion: ER stress is implicated in the pathogenesis of prediabetic and diabetic peripheral neuropathies. Studies of biochemical mechanisms of ER stress-induced peripheral nerve damage are in progress.

PS 110 Autonomic neuropathy - clinical observations

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The reproducibility of cardiovascular reflex tests is not influenced by actual glucose values in young type 1 diabetic patients
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Background and aims: The reproducibility of cardiovascular reflex tests (CRT) is well-documented, but the possible role of the current glucose levels on the heart rate and blood pressure responses in diabetic patients is unknown. The aim of our study was to analyse the CRT-s as well as continuously measured glucose values during the tests on three consecutive days in young patients with short-standing type 1 diabetes (DM).
Materials and methods: 10 young type 1 DM patients were included into the study (duration of DM: 8.2±6.7 yrs, age: 23.3±0.7 yrs, HbA1c: 8.8±0.6%; mean±SE). The five conventional Ewing CRT-s were performed on three consecutive days. During the tests the current glucose values were detected by continuous subcutaneous glucose measuring system (CGMS; Medtronic Ltd). One-way ANOVA test was applied for the statistical evaluation.
Results: The CRT results did not differ significantly between the days (mean values of the days: Valsalva ratio: 1.57-1.54-1.67; heart rate response to deep breathing: 26.4-22-25.7 beats/min; 30/15 ratio: 0.96-0.99-0.96; diastolic blood pressure response to handgrip: 24.8-19.8-23.9 mm Hg; orthostatic systolic blood pressure response: 7.7-8.9-6.3 mm Hg; AN scores: 2.7-2.4-2.5, $p>0.05$ for all tests). The subcutaneous current glucose levels did not show any statistical association with the daily results of the CRT-s. The fluctuation of glucose was not significant during the three days (mean glucose levels of the days: 7.2-9.6-5.7 mmol/l; $p>0.05$).
Conclusion: Our results confirm the high reproducibility of the cardiovascular reflexes in young type 1 diabetic patients. Data suggest that the short-term variability of cardiovascular responses is not influenced by the actual glucose levels. Our observations might indirectly support the importance of long-term glycemic exposure on the cardiovascular autonomic function in type 1 diabetes.

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Gastric neurostimulation significantly relieves symptoms of severe diabetic gastroparesis and reduces frequency of hospital contacts
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Background: In its most unremitting form diabetic gastroparesis may present with continous nausea and vomiting leading to hospitalisation for fluid substitution and blood glucose control. Fortunately the condition is often more limited, although frequently resistant to standard pharmacological and symptomatic treatment.It is, however, commonly accepted that diabetic gastroparesis, symptomatic or asymptomatic, may severely impact blood glucose control and hamper the eficiency of pharmacotherapy.
Method: Selected Type 1 diabetes patients suffering severe symptoms of diabetic gastroparesis underwent extensive clinical examination to exclude other causes of dyspepsia. All were before and after implantation of a high-frequency low-intensity gastric neurostimulator subjected to detailed clinical studies including scintigraphic gastric emptying studies, cardiac autonomic function tests, gastroscopies, visceral-biomechanical studies, 24h-antroduodenal motility testing and qualitative symptomatic testing using a validated questionnaire. Patients: Fifteen patients, 7 male and 8 female, all Type 1 diabetes, all long duration of diabetes, all presenting with an array of late diabetic complications and attending the out-patient clinic at Department of Endocrinology MEA, Aarhus University Hospital. All had suffered symptoms of diabetic gastroparesis for several years resistant to standard treatment measures and all attempts of pharmacotherapy. All were frequently admitted to hospital for this condition. A proportion of the patients had been considered to be candidates for irreversible gastrointestinal surgery.
Results: Fifteen patients have been relieved to varying degrees of their symptoms of diabetic gastroparesis. Two male patients and three female patients

are completely relieved of nausea and vomiting. Several patients demonstrate halving of admission frequency and are predominantly admitted for treatment of competing diabetic late complications. Some have not been admitted to hospital after implantation of the gastric neurostimulator, whereas they were frequently so before. Some have returned to full-time work. We were not able to demonstrate improvements in glycaemic control in this small cohorte.
Conclusion: We conclude that the patients selected and included in our study were significantly relieved of severe symptoms of diabetic gastroparesis and the frequency of hospital contacts overall were reduced.

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The association between diabetic autonomic neuropathy and microalbuminuria
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Background and aims: Diabetic autonomic neuropathy (DAN) has a negative impact on quality of life and survival in patients with type 2 diabetes (T2DM). However, as most of affected patients remain asymptomatic for a long period, it is frequently overlooked. As microvascular diseases share pathogenesis mainly originated from chronic hyperglycemia, microalbumiuria which is annually measured in patients with T2DM might be associated with DAN. We performed this study to investigate whether urinary albumin excretion (UAE) could predict diabetic autonomic neuropathy (DAN).
Materials and methods: We retrospectively reviewed records of patients with type 2 diabetes (n=953) who had received annual diabetes-related complication screening between January 2007 and June 2009. Tests for autonomic functions (AFT) measured heart rate variability during breathing, Valsalva maneuver, 30:15 ratio, blood pressure (BP) response to standing and handgrip. The results of each test were scored as 0 for normal and 1 for abnormal and assessed total score considering positive DAN larger than 2. Urinary albumin excretion (UAE) was calculated in spot urine estimating ratio of urinary albumin (mg) dividing urinary creatinine (g).
Results: The prevalence of DAN was 42.1% (n=401). Subjects with DAN had less prevalent of male and significantly higher age, longer diabetes duration and increased CAVI and IMT. On the contrary there was no difference in glycemic control status (FBS, HbA1C), hypertension, lipid profiles. Urinary albumin excretion was linearly increased according to AFT score [0 (n=176), 1 (n=221), 2 (n=194), 3 (n=79), 4 (n=31), 5 (n=3); UAE 36.1 ± 66.5, 49.7 ± 113.8, 68.3 ± 138.8, 89.9 ± 149.7, 89.8 ± 149.6, 146.1 ±224.2, 403.1 ± 386.0 mg/g, *P* for trend <0.001]. In the multivariate logistic analysis, UAE was significantly associated with DAN [odds ratio (OR) 1.002; 95% confidence interval (CI) 1.001-1003, besides age, female sex, duration, smoking status, previous CAD.
Conclusion: Elevated urinary albumin excretion was significantly associated with AFT score and a predictor for DAN in patient with T2DM. This result suggests that annual exam of UAE could additionally give information to discriminate high risk patients in DAN in addition of diabetic nephropathy.

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Effect of adjuvant influenza vaccine on systemic inflammation and cardiac autonomic function in patients with type 2 diabetes
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Background and aims: Both inflammation and impaired cardiac autonomic function are known to increase the risk of coronary events. Recent data points out a pathogenetic link between nervous autonomic system (ANS) and inflammation, but the exact terms of this relation in the clinical are still poorly defined. In this study we assessed the effect on cardiac autonomic function of the administration of an inflammatory stimulus, represented by influenza A vaccine. The effect on platelet reactivity was also assessed to better define the cardiovascular risk profile consequent to the inflammatory stimulus.
Materials and methods: A 24-hour electrocardiogram Holter recording was performed both at baseline and after 24 hours from adjuvant influenza A vaccine in 30 patients with type II diabetes mellitus (age 62±8 years, 19 men). C-reactive protein (CRP) and interleukin-6 serum levels were measured, and monocytes platelets aggregates (MPA) were assessed before and 48 hours after vaccination.
Results: Following vaccination, inflammatory cytokines, MPAs and monocyte-platelet receptor expression increased (e.g., CRP: 2.6±2.8 vs. 7.1±5.7 mg/L; p<0.0001), whereas HRV decreased (e.g., very low frequency [VLF] amplitude: 34.6±11.8 vs. 31.0±10.2 ms; p=0.002). The changes in CRP correlated with those of most HRV variables, but greater CRP increases were associated with lower HRV reductions; the most significant correlation was between changes in CRP and in SDNN(r=0.43; p=0.02) and VLF amplitude (r=0.39; p=0.03). MPA changes did not correlate with changes in CRP levels or in HRV variables.
Conclusion: In this study we show that exposition to an attenuated infectious stimulus induces, together with the expected inflammatory reaction, a relative increase of adrenergic tone in the sympatho-vagal balance of cardiac autonomic function. However, enhanced inflammatory reaction seemed to cause a vagal activation which limited the impairment of HRV and was likely finalized to antagonize the inflammatory related tissue damage. Influenza vaccination in our type 2 diabetic patients also induced increased monocyte and platelet activation that might transiently increase the risk of acute cardiovascular events after the treatment.

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Erectile dysfunction, androgen deficiency and chronic complications in male diabetic patients
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Background and aims: Erectile disfunction (ED) can be present in male diabetic patients not only induced by androgen deficiency, but also as a consequence of chronic complications.
The aim of our study was to evaluate the corelation between ED, sex-hormonal status and chronic complications in patients with diabetes mellitus (DM).
Materials and methods: 292 patients (44 T1DM/ 248 T2DM) aged between 20-75 years (mean 52.06±3.7 years) were evaluated by sex-hormonal status (DHEA , free testosterone) and by presence of chronic micro- and macrovascular angiopathy. ED was diagnosed by a score under 22 of the 5-item IIEF questionnaire. All patients with free-testosterone under 70 pg/ml were considered hypogonadic.
Results: The prevalence of ED was 84.24% in whole study group (higher in T2DM 87.5%, than in T1DM 65.9%). In patients with ED the prevalence of hypogonadism was 31.57% in T1DM and 26.73% in T2DM. From hypogonadic T2DM subjects 93.9% have ED, while in hypogonadic T1DM only 66.6% have ED (p=0.04). In older man with T2DM (over 60 years) IIEF-score was significant corelated with DHEA value r=0.57, p=0.008. We did not found any corelation between ED and macrovascular diseases. There was a significant corelation between ED and retinopathy(r=0.37, p=0.003) in T1DM and also with neuropathy (r=0.42, p=0.04) in T2DM.
Conclusion: ED is frequent in diabetic patients more associated with microvascular complications. Hypogonadic status can explain 30% of ED. In older diabetic men the severity of ED is related to low DHEA-value.

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Erectile dysfunction in diabetics and non-diabetics due to macrovascular lesions
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Background and aims: To study incidence of erectile dysfunction (ED) in the diabetics and non-diabetics by macrovascular lesions.
Materials and methods: We examined 601 male patients with type 1 and type 2 diabetes mellitus and 413 non-diabetic persons aged from 20 to 70 in both groups. The examinees were differentiated by presence of ischaemic heart disease (IHD), myocardial infarction, cerebral circulation disturbance and stroke as per medical history, clinical-laboratory investigations and instrumental data. They were inquired with the International Erectile Function Index questionnaire.
Results: ED incidence in the diabetics with IHD (85.4+2.65%) was 18.7% higher (P<0.001) than in the diabetics without macrovascular lesions (66.7+2.5%). Similar tendency in ED incidence was observed in the non-diabetics, thus in persons with IHD but without DM the incidence (79.2+8.29%) was 45.3% higher (P<0.001) than in the non-diabetics and macrovascular

lesions (33.9+2.44%). Comparison of ED incidence in groups of the non-diabetics without macrovascular lesions with ED incidence in the group of the diabetics without macrovascular lesions (66.7+2.5%) showed that it was 32.8% higher (P<0.001) than in the non-diabetics without macrovascular lesions (33.9+2.44%). ED incidence in the diabetics with myocardial infarction (85.0+5.6%) was 18.3% higher (P<0.001) than in the diabetics without macrovascular lesions (66.7+2.5%). Similar tendency was observed in the non-diabetics with and without myocardial infarction. Thus, in 3 of 3 non-diabetics with IHD (100+0%) ED of moderate severity was found in one person, the most severe one being registered in two, while in the non-diabetics without IHD the parameter was 33.9+2.44% (P<0.001). ED incidence in the diabetics with cerebral circulation disturbances was 83.3+7.6%. It is 16.6% lower than the parameter in the non-diabetics. The highest ED incidence could be seen in the diabetics after the stroke (100%). In the group of the non-diabetics without macrovascular lesions the stroke was registered in one patient with ED of intermediate severity.As a whole in both diabetics and non-diabetics with macrovascular lesions higher ED incidence can be observed than in patients without the lesions. Thus, in the diabetics and non-diabetics without the lesions ED incidence was 66.7+2.5% and 33.9+2.44% respectively. At the same time in the diabetics and non-diabetics with the macrovascular lesions ED incidence was found 85.9+2.1% and 84.2+5.9%, respectively.
Conclusion: Thus, in patients with macrovascular lesions (IHD, myocardial infarction, cerebral circulation disturbance and stroke) regardless of DM presence ED incidence is higher than in persons without the lesions in question.

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Sexual dysfunction in pre-menopausal normal and diabetic women; clinical, psychologic, cardiovascular, and neurophysiological correlates
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Background and aims: Female Sexual Dysfunction (FSD) is frequent in women with diabetes mellitus or metabolic syndrome. Aim of this study was to analyze clinical, cardiovascular, and neurophysiological correlates of FSD.
Materials and methods: We evaluated 66 pre-menopausal women, 42 healthy and 24 with diabetes (8 T1DM, 16 T2DM, duration 9.6±1.91 y, with no clinical micro-, macro-angiopathic, or neurologic complications), through administration of Female Sexual Function Index questionnaire (FSFI). In all women we studied: physical activity, smoking habits, parity, weight, BMI, waist circumference (WC), Beck Depression Inventory (BDI), Diabetic Somatic Neuropathy Score (DSN), endothelial-mediated blood flow, ECG (for heart rate and Qtc, indexes of sympathetic activity), intima-media thickness (IMT), insulin, fasting glucose, HOMA-IR index, fibrinogen, cholesterol (total, HDL-, LDL-), triglycerides, HbA1c, HS-PCR, electromyography (amplitude and conduction of peroneal, posterior tibial, and sural nerves).
Results: Diabetic women differed from healthy women for BDI, fasting glucose, triglycerides, HbA1c, nerve conduction (p<0.05 to p<0.001), and at FSFI for orgasm (p<0.05). In healthy and diabetic women considered together, FSFI score was directly correlated with physical activity (r = .266), and with peroneal nerve amplitude (r = .289), and inversely with parity (r = .298), BDI (r = .483), SDN (r = .439), IMT (r = .329), (p<0.05 to p<0.01).
Conclusion: These data indicate that: 1) FSFI is reduced in reasonably healthy diabetic women even in the absence of complications; 2) FSFI correlates with psychologic, neurophysiologic, and cardiovascular parameters. Longer observation periods are required to evaluate FSFI as a possible risk factor for cardiovascular events.

PS 111 Autonomic neuropathy - blood pressure and heart

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Heart rate variability during the night is related to hyperglycaemia in patients with type 2 diabetes
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Background and aims: Heart rate variability (HRV) represents a non-invasive technique that allows a detailed study of the cardiac autonomic nervous system, by assessing the spontaneous fluctuations of heart rate. HRV is reduced in patients with diabetes and is considered a risk factor for cardiovascular mortality. Aim of the present study was to assess the relationship between glycaemia (measured by continuous glucose monitoring subcutaneously [CGMS]) and HRV (measured by continuous ECG monitoring) simultaneously in patients with Type 2 diabetes (T2D).
Materials and methods: A total of 31 (20 males) T2D patients (mean age [±SD] 56.2±9.9 years, diabetes duration 5.5±4.2 years), treated with oral antidiabetic agents, underwent ECG recording and CGMS, simultaneously and continuously, for 48 hours. HRV was calculated by frequency and time domain analysis. A separate analysis was performed regarding HRV during the day and night period (11.00 pm to 08.00 am). CGMS was performed by a needle electrode placed subcutaneously in the abdomen, acquiring data every 5 min (288 measurements/day), with a microdialysis system.
Results: There was no correlation between HRV and HbA1c or 48-h mean plasma glucose. Weak negative correlations were found between HRV indices and hyperglycemia, expressed as the area under the curve (AUC) of glucose values above 180 mg/dl (AUC_{G180}). Strong negative correlations were observed, however, between HRV indices during the night period and 48-h AUC_{G180}. No such correlations were observed during the day time (Table). HRV, in both frequency and time domain analysis, was significantly higher during the night period than during the day (P values for all indices < 0.001).
Conclusion: HRV during the night period is negatively correlated with 48-h hyperglycemia, while no such association is observed during the day. It may be hypothesized that this finding reflects a relation between HRV and hyperglycemia, being unmasked during sleep, when HRV is not affected by day-time activities.

Linear regression of indices of HRV during night and AUCG180 (adjusted for duration of diabetes)

Indices of HRV	β-coefficient	β-coefficient	P	P
A. Time domain analysis	**Day**	**Night**	**Day**	**Night**
Percent of differences between normal-to-normal RR intervals >30 ms (PNN-30)	-0.19	**-0.40**	0.30	**0.03**
Percent of differences between normal-to-normal RR intervals >50 ms (PNN-50)	0.03	**-0.48**	0.97	**<0.01**
Root mean square of successive normal-to-normal RR interval difference in ms (RMSSD)	-0.05	-0.36	0.80	0.052
B. Frequency domain analysis				
Total power of HRV (TP)	-0.1	**-0.38**	0.87	**0.03**
High-frequency domain of HRV (HF)	-0.25	-0.38	0.18	0.051
Low-frequency domain of HRV (LF)	0.10	-0.20	0.61	0.29

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Heavy smoking blunts the circadian rhythm of heart rate variability in patients with type 2 diabetes
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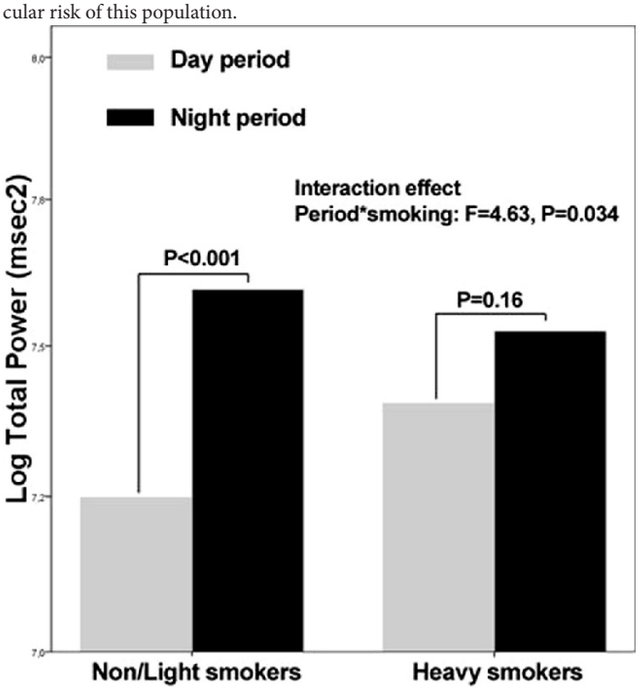
Background and aims: Autonomic nervous system control of the cardiovascular system has a distinct circadian rhythm and this may be an important mechanism underlying the diurnal distribution of cardiac events. Heart rate variability (HRV) represents a non-invasive technique that allows a detailed

study of the cardiac autonomic nervous system, by assessing the spontaneous fluctuations of heart rate. HRV is reduced in patients with T2D, while it is known that smoking also decreases HRV. Aim of the present study was to assess the effect of smoking on circadian rhythm of HRV in patients with Type 2 diabetes (T2D).
Materials and methods: Sixty three consecutive non-smokers and 35 consecutive smokers, attending the diabetes outpatient clinic of a University Hospital, treated with oral antidiabetic agents, underwent continuous ECG monitoring for 24 hours. HRV was calculated by frequency and time domain analysis. A separate analysis was performed regarding HRV during the day and night period. Smokers were further divided into heavy smokers (n=29), defined as being exposed to ≥10 pack-years (py), and light smokers, being exposed to <10 py.
Results: Smokers were younger (mean age: 54.4±9.6, vs. 61.5±8.8 years, P<0.01) and had a shorter duration of diabetes (median duration: 4 vs. 7 years: P=0.054). Smokers had higher HRV indices than non-smokers, although statistical significance was attained only for high and low-frequency domain analysis during the day. Both smokers and non-smokers had higher HRV during the night (all P values <0.05). Heavy smokers, however, showed a statistically significant increase in HRV during the night period only for some domains of HRV analysis (high-frequency domain (HF), low frequency domain (LF) and percentage of differences between normal-to-normal RR intervals >30 ms [PNN-30]). In one-way repeated-measures ANOVA, heavy smoking was associated with a blunted increase of HRV during the night (Figure). This finding was consistent for all indices of HRV, both in frequency and time domain analysis.
Conclusion: Smoking >10 pack-years blunts the circadian rhythm of HRV in patients with T2D. This effect may be implicated in the increased cardiovascular risk of this population.



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Autonomic neuropathy as the cause of the QTc dispersion in diabetes type 2
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Background and aims: The increase of the QT_c dispersion reflects the electrical instability of the diabetic heart. It is an important risk factor of sudden death caused by ventricular fibrillation. It occurs in persons with diabetes mellitus more frequently than in the general population. A closer determination of the pathogenetic factors involved in QT_c dispersion should improve the preventive measures.
Materials and methods: For the study, a) a general cohort of 185 males with diabetes mellitus type 2 without symptoms of ischemic heart disease, aged 57.6±7.1 years, and b) 52 persons without diabetes mellitus and without symptoms of ischemic heart disease - males, aged 58.8±7.2 years - were qualified. The following investigations were conducted on those under study: standard clinical examination, indices of metabolic compensation of diabetes mellitus (daily glycaemia profiles, HbA_{1c}, lipid profile), serum potassium, natrium calcium and magnesium levels, resting ECG, exercise test according to Bruce protocol and the battery of tests for the autonomic innervation of the heart according to Clarke protocol. The subgroup of diabetes mellitus type 2 with a diagnosis of autonomic neuropathy was composed of 84 subjects, males aged 56.8±6.5 years. The QT length was derived from the average of readings from a 12-leads ECG and calculated with the Bazett's formula: $QT_{cd} = QT_{c\,max} - QT_{c\,min}$.
Results: In diabetes mellitus type 2 under study, without clinical symptoms of ischemic heart disease, the statistically significant (p<0.001) increase of QT_c - to 41±21 ms - in comparison with the QT_c in the control group, 22±11 ms - was revealed. In the subgroup of diabetes mellitus type 2 with the silent ischemia of the heart, the QT_c values were similar to those in the subgroup of diabetes mellitus type 2 without such ischemia. This was in contrast to the finding of a correlation between the presence of autonomic neuropathy and the increase of the QT_c dispersion.
Conclusion: In persons with diabetes mellitus type 2 without clinical symptoms of heart ischemia the disturbances of the ventricular repolarization as manifested by an increase of the dispersion of QT_c, are correlated with the markers of autonomic neuropathy. Such correlation was not found in the subgroup without symptoms of heart ischemia or with the asymptomatic ischemia of the heart without autonomic neuropathy. Therefore, in cardiological care for diabetic persons, early testing for autonomic neuropathy should be included as a matter of routine.

Diabetes mellitus type 2 no clinical heart ischemia no silent heart ischemia	Diabetes mellitus type 2 silent heart ischemia present
24±11 ms	23±14 ms (p>0.05)
Diabetes mellitus type 2 no clinical symptoms of heart ischemia autonomic neuropathy present	Diabetes mellitus type 2 no clinical symptoms of heart ischemia autonomic neuropathy absent
53±18 ms	38±2 ms (p<0.05)

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The prognostic value of sudomotor function for the development of cardiovascular disease and retinopathy: a 3-year follow up of diabetic patients
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Background and aims: The aim of the present study was to evaluate the risk for vascular morbidity or death and retinopathy in relation to an abnormal response to a commercially available test of distal autonomic function (Neuropad®).
Materials and methods: To that end, we performed a 3-year follow- study of type 2 diabetic patients attending the outpatient clinic. A total of 348 adult patients were evaluated for the degree of retinopathy and levels of HbA1c, blood pressure, serume creatinine and proteinuria. Somatic sensory neuropathy was documented using the Neuropathy Disability Score, and autonomic neuropathy (sudomotor neuropathy) was determined on the sole of the foot using the Neuropad® response including time to colour change. Patients were divided into two groups: those without and with new vascular morbidity and causes of death which were registered by one and the most severe event only.
Results: Initially there were 348 patients with type 2 (159 male, mean age 62.0±8.8y; mean disease duration 14.8±8.6y). NDS was >5 (abnormal) in 83 (23.9%) and no change in Neuropad colour by 10 minutes was observed in 103 (29.6%). Significant direct correlations were observed between an abnormal Neuropad test and age (r_s=0.31, p<0.001), waist circumference (r_s=0.17, p=0.003), BMI (r_s=0.25, p<0.001); significant negative correlations were observed with height (r_s=-0.17, p<0.001) and serum HDL cholesterol (r_s=-0.13, p=0.003).The data represent values taken 3±1years (mean±SD) after initial evaluation. Twenty-two patients developed atherosclerotic vascular disease, i.e. myocardial infarction (n=9), cerebrovascular disease (n=9), or amputation (n=4), and 19 died. The observed annual mortality rate was 18.4/1000 compared to an expected rate of 12.6/1000 for the general population with corre-

sponding age and sex. Male gender (62.8% vs. 45.6%, p=0.04), age (68.8±6.1y vs. 61±8.1y, p<0.001), high BMI (30.3±4.9 vs. 27.1±2.3, p<0.01), high triglycerides (3.1±0.9 vs. 2.0±0.7mmol/l, p<0.01), low HDL cholesterol (1.06±0.3 vs. 1.16±0.17mmol/l, p<0.01) and time to complete Neuropad colour change (22.6±5.8mins vs. 9.1±6.7mins, p<0.01) were associated with vascular disease or death. Time until total colour change was found to be a prognostic marker for the development of vascular disease and death in patients treated with insulin at baseline (23.3±5.9mins vs. 8.7±4.7mins, p<0.01), whereas this was not the case in patients treated with oral agents at baseline (11.3±4.5mins vs. 7.4±5.6mins, NS). However, insulin treatment per se was not associated with an icreased mortality or morbidity. Time until total colour change was correlated with incidence of prolipherative retinopathy regardless of diabetes treatment (17.3±6.9mins vs. 9.1± 5.4mins, p<0.01).
Conclusion: This study showed that abnormal test of sudomotor function was a prognostic factor for vascular morbidity and death in type 2 diabetic patients treated with insulin but not in patients treated with oral agents.

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99mTc - Myoview gated - SPET and heart rate variability measurement in detection of early cardiovascular changes in diabetic patients
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Background and aims: Chronic metabolic alteration in diabetic disease implicates the changes in myocardial perfusion and autonomic nervous system. Risk of myocardial infarction is 3 times higher compared to whole population. We decided to examine diabetic patients without history of cardiovascular disease for presence of cardiac autonomic cardiomyopathy and compare the results from standard examinating methods (treadmill test and echocardiography) to 99mTc - Myoview gated - SPET findings.
Materials and methods: We examined 47 patients, 20 individuals with T1DM (13 men, 7 women), average age 37 ± 12,7 years and 27 individuals with T2DM (14 women, 13 men), average age 60 ± 9,2 years. Written consent was obtained from all patients prior the study. In all patients we provided echocardiography and battery of Ewing´s testing combined with heart rate variability (HRV) examination. Thereafter patients underwent treadmill test and stress 99mTc - Myoview gated-SPET. Vascular and metabolic determinants were recorded. Colected data were analysed using nonparametric statistic methods.
Results: Treadmill test was negative in all patients. Echocardiography revealed diastolic dysfunction in 10 % of T1DM and 11 % of T2DM, no patient had systolic dysfunction. Scintigraphy confirmed hypoperfusion in 35 % T1DM (p = 0,01) and in 60 % T2DM (p = 0,001). Diagnose of cardiac autonomic neuropathy based on Ewing´s testing and examination of HRV was estimated in 60 % of T1DM patients (p = 0,001) and 77 % of T2DM patients (p = 0,001). In T1DM group we found association between cardiac autonomic neuropathy (CAN) and frequency of hypoglycaemia (p = 0,04) and trend with duration of diabetes mellitus (p = 0,069). We did not find any correlation among all examined parameters in paciwnts with T2DM.
Conclusion: We revealed high incidence of cardiovascular changes characterised with myocardial hypoperfusion and cardiac autonomic neuropathy among diabetic patients while treadmill test and echocardiography showed negative finding. Therefore we suggest that heart disease develops in diabetic patient many years undetected and advanced preventive arrangement is needed.

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The effect of cardiovascular autonomic neuropathy on progressing chronic heart failure in patients with type 2 diabetes mellitus
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Background and aims: Chronic heart failure (CHF) in patients with diabetes mellitus (DM) is a frequent and with poor prognosis. Cardiovascular autonomic neuropathy (CAN) is a common complication of DM, associated with increased mortality and myocardial ischemia. Aim: to study the influence of CAN on progressing CHF in patients with Type 2 DM.
Materials and methods: Fifty - nine patients with CHF and Type 2 DM with CAN (Group A) and 29 patients with CHF and Type 2 DM without CAN (Group B) were enrolled in the study. Both groups were comparable in age, gender, BMI, stage of CHF, there were no patients with acute myocardial infarction (MI) or advanced diabetic nephropathy. The 6 minutes walking test, echocardiography, and evaluation of the heart rate variability (HRV) by 5 minutes ECG monitoring were performed. The study lasted for a year.
Results: Within a year the MI has developed in 10 patients of group A and 1 patient of group B (p = 0,049). The patients of group A, who have suffered MI during supervision, were characterized by especially low parameters of standard deviation of all NN-intervals (SDNN). In particular, of patients of group A with the level SDNN <20 ms 8 patients (32 % from all patients with level SDNN <20 ms) have suffered MI, on the contrary, in patients with parameter of SDNN within the limits of 20 - 33 ms MI has developed only in 2 (p = 0,016). In both groups severity of CHF has increased, but in group A progressing CHF was more expressed. It has been demonstrated by worsening of clinical features, decrease of the left ventricle function. The decrease of the ejection fraction of the left ventricle, in 1 year was significantly higher in group A compared to group B (Median [25th; 75th percentiles] 4 [3; 7] v 2 [2; 5]), p = 0,019). In a year a decrease according to 6 minutes walking test was significantly higher in group A than in group B (Median [25th; 75th percentiles] 50 [20; 80] v 15 [4; 45], p = 0,023).
Conclusion: The risk of myocardial infarction of the patients with combination of Type 2 diabetes mellitus and chronic heart failure was higher in the presence of cardiovascular autonomic neuropathy. The level of SDNN lower than 20 ms is the precursor of a negative cardiovascular prognosis. Cardiovascular autonomic neuropathy causes the progressing chronic heart failure in the patients with Type 2 diabetes mellitus.

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Autonomic function and circadian blood pressure changes in patient with impaired glucose tolerance
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Background and aims: Diminished circadian blood pressure changes may be present in patients with diabetes mellitus, in partly related to autonomic neuropathy.
Materials and methods: The aim of our study was to evaluate whether or not a similar connection may exist in patients with impaired glucose tolerance (IGT). We examined 46 patients with IGT (age: 53.04±11.10 years, fasting blood glucose 5.40±0.57 mmol/l; 120 min blood glucose: 8.61±1.01 mmol/l; HbA_{1c}: 5.97±0.38 %; x±SD) while 45 healthy subjects (age: 55.84±11.41 years) served as controls. Cardiovascular autonomic neuropathy was detected by the five standard tests of cardiovascular function. Systolic and diastolic blood pressure (BP) means just as systolic and diastolic diurnal indices were assessed by 24-hour ambulatory blood pressure monitoring.
Results: Significant differences were found between IGT and control subjects regarding the following parameters: beat-to-beat variation (11.90±5.70 vs. 19.50±4.10 beats/min; p=0.0001), Valsalva ratio (1.23±0.25 vs. 1.46±0.22; p=0.0001) and increase of diastolic blood pressure during sustained handgrip (19.90±7.90 vs. 23.70±6.10 mmHg; p=0.012) were lower, while postural systolic blood pressure changes (4.37±5.70 vs. -0.60±2.00 mmHg; p=0.0001) were higher in patients with IGT versus control group. Both systolic (9.58±7.59 vs. 13.70±5.90; p=0.003) just as diastolic (15.50±10.71 vs. 18.93±7.13; p=0.095) diurnal indices were lower in IGT subjects compared to controls. The 24-hour systolic (121.80±5.49 vs. 116.75±9.85; p=0.048) and diastolic (74.95±3.86 vs. 71.61±6.10; p=0.008) blood pressure means were significant higher in IGT versus control group.
Conclusion: Our data suggest that the presence of cardiovascular autonomic neuropathy is associated with diminished circadian blood pressure changes in patients with impaired glucose tolerance.

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Influence of vagosympathetic changes on arterial stiffness in diabetic and obese patients
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Background and aims: Arterial stiffness is often increased in patients with diabetes or obesity. Several data suggest the role of vagosympathetic changes

in arterial hypertension. The aims of the present study were to examine the role of cardiovascular vagosympathetic changes on peripheral and central arterial stiffness in patients with type 2 diabetes or obesity.
Materials and methods: We included 207 patients (142 hypertensive) with type 2 diabetes (T2D) and 68 non diabetic obese (22 hypertensive) patients. Arterial stiffness was evaluated by measuring carotid to femoral pulse wave velocity (PWV) (Complior®) and by brachial and finger pulse pressure (PP), vagosympathetic activity by spectral analysis of heart rate (HR) and blood pressure (BP) variations (Finapres ; HF : high frequency and LF : low frequency peak).
Results: In T2D patients as compared with obese patients, PWV ($p < 0.01$), brachial ($p < 0.001$) and finger ($p < 0.05$) pulse pressure (PP) were significantly higher and HF-HR ($p < 0.05$) lower, and this was confirmed after age and blood pressure adjustment. In T2D patients, PWV correlated significantly with age, systolic BP, LF-systolic BP, microalbuminuria and duration of diabetes and negatively with creatinine clearance and HF-HR and was significantly higher in the patients with peripheral neuropathy or peripheral vascular disease ($p<0.05$ to <0.001) ; brachial and finger PP also correlated significantly with all these parameters. In obese patients PWV and similarly brachial and finger PP correlated with age, systolic BP, microalbuminuria and negatively with creatinine clearance and HF-HR ($p = <0.01$ to < 0.001). In multivariate analyses including these parameters as independent variables, PWV was significantly associated with age both in T2D and obese patients, and with systolic BP and BMI in T2D patients; brachial PP correlated with age in both groups; finger PP correlated with HF-HR, LF-systolic BP and age in T2D patients ($p<0.001$, 0.01 and 0.05) and with HF-HR and systolic BP in obese patients ($p <=0.01$for both).
Conclusion: These data strongly suggest that in T2D and non diabetic obese patients, vagosympathetic changes play a major role in stiffness of small but not large peripheral arteries.

PS 112 Diabetic foot - clinical observations

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Diabetes related amputation incidence in an inner city multiethnic population in the UK before and after the introduction of multidisciplinary diabetes foot care, 2000-2008

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Background and aims: Previous studies have suggested that appropriate multidisciplinary diabetes care can reduce the incidence of lower extremity amputations. According to the U.K. National Centre for Health Development Data for 2003-04, our inner city area in London was reported to have a higher number of diabetic amputations than the national average and amongst the highest within London. The Combined Foot Clinic was established in 2005 to offer multidisciplinary diabetes care for patients with diabetes related foot ulcers. We introduced care pathways and protocols for managing diabetic foot ulcers and raised awareness of the diabetic foot among primary care healthcare professionals through an educational programme. The aim of this study was to assess changes in diabetes-related lower extremity amputations in a multiethnic population over an 8-year period following the introduction of a multidisciplinary foot team. We reviewed the diabetes care of patients with diabetic amputations prior to, during and after admission.
Materials and methods: Our hospital covers a multiethnic population in an inner city area of London. All diabetes related lower extremity amputations in our insitution between 2000 and 2008 were identified via coding. Data were collected retrospectively and were analysed for the period before (2000 - 2005) and after (2006 - 2008) the introduction of the multidisciplinary foot team.
Results: From 2000 to 2005, 104 amputations in 64 patients (mean age 68.6 years, 70% males and 30% females, mean duration of diabetes 15 years) were identified with an incidence of 17.3 amputations/year. There were 38 major amputations (6.3/year) and 66 minor (11/year). Perioperative mortality was 6.6 %. From 2006 to 2008, 44 amputations in 33 patients (mean age 70.1 years, 72% males and 28% females, mean duration of diabetes 15 years) were identified with an incidence of 14.7 amputations/year. There were 9 major amputations (3/year) and 35 minor (11.7/year). Perioperative mortality was 4.6 %. During admission, between 2006 and 2008, 75 % of patients had HbA1c (median HbA1c 7.3%) compared with 41% between 2000 and 2005 (median HbA1c 7.9%). During admission, 81% of the patients were reviewed by Podiatry/Tissue Viability team compared with 40% for the previous period. After discharge, the proportion of patients who had follow-up by the Diabetes team increased from 62% to 73% and by Podiatrist from 43% to 80%. Overall, considerable improvement in the involvement of diabetes team and podiatry services was reported. Improvement in foot care services after 2005 led to a 53 % reduction in the incidence of major diabetes related amputations from 6.3/year to 3/year and a 15 % reduction in total diabetic amputations in our population from 17.3/year to 14.7/year. Overall, the incidence of major diabetes related amputations in our area has decreased to 1.2/100,000 general population/year and of total amputations to 5.8/100,000 population/year and is much lower than the national incidence of 3.4/100,000/year and 8.7/100,000/year respectively. Our perioperative (1-month) mortality rate is 4.6 % compared to 6.6 % nationally.
Conclusion: We demonstrated considerable improvements in outcomes of diabetes related amputations following improvements in foot care through multidisciplinary team work.

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Changes in the incidence of lower extremity amputations in people with and without diabetes in England between 2004 and 2008

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Background and aims: During the last decade, there was a consistent upward trend in the number of non-traumatic lower extremity amputations in people with diabetes in England. However, there is a lack of national data on the incidence of diabetes-related amputations. The aim of this study was to describe recent trends in non-traumatic amputations in people with and without dia-

betes and estimate the relative risk of amputations in diabetes between 2004 and 2008 in England.
Materials and methods: We identified all patients who underwent any non-traumatic amputation in England between 2004 and 2008 using national hospital-activity data from all NHS hospitals. Age- and sex-specific incidence rates were calculated using the total diabetes population in England every year.
Results: Overall amputation rates (minor and major combined) decreased by 9.1%, from 27.5 per 10,000 persons with diabetes in 2004 to 25.0 per 10,000 persons with diabetes in 2008. The rate of minor LEAs fell from 15.7 to 14.9 per 10,000 persons with diabetes and major LEA rates decreased from 11.8 to 10.2 per 10,000 persons with diabetes. The absolute reductions in minor and major LEA rates were slightly greater in men with a decline from 19.9 to 18.3 per 10,000 persons with diabetes compared to a fall from 7.6 to 6.7 per 10,000 persons with diabetes in women. Poisson regression analysis showed no statistically significant change in amputation rates over time in people with diabetes after adjustment for age, sex, level of amputation and year (0.97 decrease per year 95% CI 0.92-1.02, p=0.374). Amputation rates (minor and major combined) decreased from 13.6 per 100,000 persons without diabetes in 2004 to 11.9 per 100,000 persons without diabetes in 2008. Minor and major LEA rates showed a decline from 5.9 to 5.0 100,000 persons without diabetes and from 7.7 to 6.9 100,000 persons without diabetes, respectively. Poisson regression analysis showed that the decline in non-diabetes-related LEAs did not reach statistical significance (0.96 decrease per year, 95% CI 0.93-1.00, p= 0.07). The relative risk of a person with diabetes undergoing a LEA increased slightly from 20.3 to 21.2 when compared with persons without diabetes between 2004 and 2008.
Conclusion: This national study suggests that incidence rates of lower extremity amputation in people with diabetes England remained unchanged between 2004 and 2008. The increased relative risk of amputation among persons with diabetes compared to those without appears to have marginally increased during the study period although this might be explained by decreased risk among people without diabetes.
Supported by: European Community

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High prevalence of limb amputation in people with diabetic foot syndrome
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Background and aims: The diabetic foot syndrome is a preventable long-term complication of diabetes mellitus. One of the St. Vincent targets from 1989 was a 50% reduction in amputation. This target is generally not reached. But to show the prevalence and incidence of limb-amputation is difficult because complete epidemiological data do not exist. The aim of the study was to show the prevalence of limb-amputation in people with diabetic foot syndrome and the mean survival time after the first diagnosis in Germany.
Materials and methods: Anonymous data were obtained from the prospectively compiled IMS Disease Analyzer database. All patients with a first diagnosis of diabetic foot syndrome between January 1, 2001 and December 31, 2005 were included. Patients were required to have continuous data for at least 5 years after the first diagnosis of diabetic foot syndrome (ID). The maximum observation period was 10 years. The primary outcome measure of the study was disease-free survival following a diagnosis of diabetic foot syndrome. The documentation of an amputation [ICD10-Codes: Z894-899] indicated the end of disease-free survival. Kaplan-. Meier plots were generated to analyse the probability of amputation and the amputation-free survival time.
Results: A total of 4,068 patients with diabetic foot syndrome were included in the analysis. The overall mean age at diagnosis was 64.9 (SD: 11.9) years, 39.2% were female, the middle HbA1c was 8.05% (SD: 2.24) and 13.1% of the patient with DFS has a polyneurpathy and 12.1% has peripheral angiopathy. 3,407 patients were in 697 general practitioner practices and 661 patients were in 57 diabetology practices. Within observation time 737 (18.1%) patients had a limb-amputation: 45.2% an amputation of foot and ankle or toes, 32.7% of leg at or below knee 18.1% of leg above knee and 4.0% amputation of both lower limbs. Limb-amputations were registered among 16.6% in general practitioner practices and 26.0% in diabetology practices, 20.2% in male and 15.5% in female patients. The mean amputation-free survival time was 7.8 years (SD: 17.2 days).
Conclusion: Results from this analysis of a large representative German database show, that the prevalence of limb-amputation in people with diabetic foot syndrome is high. Screening methods to find out high risk foot problems and appropriate education in protective foot care for people with diabetes mellitus are established but obviously not effective enough. More long lasting avoiding methods are necessary.

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Cost of an episode of diabetic foot ulcer in Spain
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Background and aims: The economic burden of diabetic foot is substantial, due to long term treatments, high recurrence rate and high risk of lower extremity amputation associated. In addition, diabetic foot ulcers cause high disability and decrease the health related quality of life. The objective of this study was to obtain data concerning resource consumption in patients with diabetic foot syndrome and to estimate the direct sanitary costs per patient associated to an episode of non-complicated superficial full-thickness neuropathic ulcer of diabetic foot in Spain.
Materials and methods: This is an observational, retrospective and multicenter design. Diabetics with non-complicated superficial neuropathic ulcer of at least 10 cm2 without lower extremity ischemia, and with the study ulcer completely resolved due to wound healed or amputation. Ischemic or neuroischemic ulcers or with foot infection at the time of inclusion were excluded. The study period for each patient was the duration of the complete selected ulcer episode. Collection of study data was performed in an unique visit. The estimation of costs was performed by identification and subsequent quantification of health resources used in the treatment of the diabetic foot episode, assigning a specific unitary cost to each of these resources. Analysis was carried out according to the perspective of the National Health System, taking into account direct sanitary costs, exclusively. Results are shown as total cost per ulcer episode and expressed in Euros (€) from year 2009. The study was performed from a hospital perspective.
Results: The average cost per patient with an episode of diabetic foot ulcer in Spain is estimated at 12525.12€. Only direct sanitary costs have been considered and not the indirect costs related to the loss of productivity of patients and caregivers. The hospital admissions, topical ulcer care and surgery represent the highest percentages of the direct costs, 50.4%, 19.2% and 22.8% respectively. In our study the average cost of amputations as unitary resource cost, and considering the Diagnosis-related groups values, was estimated as 7.730,14€. Taking into account the percentage of patients in the sample that suffered an amputation, this cost represent 1649.49€ per amputation (surgery procedure only) and ulcer episode. In consequence, our study could be underestimating the cost of amputations, one of the costs with highest impact in the average cost per patient.
Conclusion: Diabetic foot is one of the complications of diabetes mellitus that produces a high economic impact in the health systems. In consequence, after estimation in this study and others published in different countries, of the costs that its management involves, it would be necessary to carry out primary and secondary prophylaxis programs in order to decrease the occurrence of diabetic foot ulcers in patients with diabetes. Additionally, it would be necessary to establish an adequate management of the lesion including treatments for accelerating the healing process and cures at home in order to shorten hospital stays. By doing this, the decrease in health related quality of life of these patients would be avoided, as well as the risk of losing the extremity and also the use of the high number of sanitary resources its treatment involves.

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Comparison of outcome of diabetic foot ulcer treatment in 3 different centres of North West England
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Background and aims: Diabetic foot ulcers are managed at different centres as per local protocol, which may vary from place to place depending on availability of local expertise. This may affect the outcome of ulcers. To assess this

we retrospectively studied the outcome of 50 diabetic foot ulcers that presented to 3 different centres in the North West England. The aim of this study was to assess outcome of diabetic foot ulcers in these centres.
Materials and methods: We retrospectively analysed the clinic notes of first 50 patients that attended 3 diabetic foot clinics of North West England from 1st January 2008. The list of patients that presented to diabetic foot clinic was obtained from the hospital system and their notes retrieved. Data was collected in pre-designed form. If needed other hospital IT systems were checked.
Results: Case notes of 148 patients (Mean age 66.3 +/- 12.9 years & 33.8% Females) were studied. Centre A had 46 patients (Mean age 68.7 +/- 13.2 years & 32.6% Females), Centre B had 51 (Mean age 66.0 +/- 11.4 years & 23.5% Females) and Centre C had 51 (Mean age 64.6 +/- 14.0 years & 45.1% Females) patients. There was no difference (p >0.05) in age and sex of patients between these centres. Similarly there was no difference in HbA1c, total cholesterol serum creatinine and blood pressure in patients attending these centres. Table 1 shows the results comparing these 3 centres, which clearly shows improved outcome in Centre B.
Discussion: Our study shows that there was significantly more proportion of ulcers healed by 12 weeks in Centre B, which is due to extensive use of cast and higher proportion of neuropathic ulcers. This trend of improved healing continued for 52 weeks of the study. There was no difference in the presence of infection, proportion of osteomyelitis confirmed on X-ray and amputation rate between these centres. There was a trend for reduced mortality in centre C that could be due to more proportion females in that centre.
Conclusion: This study confirms that there is variable outcome of diabetic foot ulcers even within a small geographical area. This is mainly due to variable use of cast. Similar comparative studies are necessary in order to compare quality of care provided to patients with diabetic foot ulcers.

Table (1): comparison of centre A, B, and C for different aspects of the outcome

Parameter	Centre A	Centre B	Centre C	P value
Healing by 12 weeks	34.8%	47.1%	19.6%	0.01
Healing by 24 weeks	41.3%	68.6%	47.1%	0.06
Healing by 36 weks	60.9%	76.5%	56.9%	0.09
Healing by 52 weeks	69.6%	86.3%	70.2%	0.09
Total Death by 52 weeks	4%	17.6%	17.3%	0.06
Use of Cast	23.9%	72.5%	9.8%	<0.001
Presence of Infection	56.5%	47.1%	62.7%	> 0.05
Osteomyelitis in X ray	34.8%	19.6%	19.6%	> 0.05
Neuropathic ulcers	45.7%	74.5%	52.9%	<0.01
Amputation	17.9%	15.7%	7.8%	> 0.05

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Diabetic foot screening: an observational study in a population of diabetics in Forli' (Northern Italy)
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Background and aims: Diabetic foot problems are among the most serious and costly complications of diabetes. Epidemiologic reports indicate that over one million amputations are performed on people with diabetes each year. A majority of these amputations are preceded by ulcers. Despite being the most serious complications of diabetes, foot complications can be effectively prevented. Several studies show how the identification of patients at high risk of ulcer and the subsequent education of these patients represent an important step to prevent this complication.
Subjects and methods: In April 2006 in our department we start a diabetic foot screening for all diabetics followed in our clinic, in a managed care organization, in order to intensify primary prevention. Until October 2008 we evaluated 920 patients affected by diabetes (mean age 67.4±12.2 yrs, mean duration of diabetes 10.5±10.8 yrs, mean HbA1c 7.58±1.5 %). Every patient underwent accurate anamnesis, foot examination, ABI and VPT measurement, Semmes-Weinstein monofilament perception, HbA1c measurement. Patients were then stratified into 4 classes of risk according to diabetic foot international guidelines (class 0: absence of neuropathy; class I: presence of neuropathy; class II presence of neuropathy and vasculopathy and/or deformity; class III: previous ulcer or amputation).
Results: Deformities were present in 250 diabetics (27.1%), onychomycosis in 240 (26%), hyperkeratosis in 310 (33.6%), ocycochriptosis in 28 (3%). In 68 subjects we found an incidental lesion or prelesion (7.4%). 435 diabetics (47.2%) showed VPT ≥25. Monofilament was not detectable in 184 patients (19.9%). In 75 patients (8.1%) we found ABI<0,9 while in 41 (4.4%) ABI was >1,3. ABI was undetectable in 90 subjects (9.7%). Of all patients screened only 156 (17%) used proper shoes. In the table we summerised data of the different classes of risk.

Class of Risk	0	I	II	III
N° (%)	388 (42)	202 (22)	241 (26)	89 (9)
Age (yrs)	61.3±12.1	70.9±9.9	70.4±11.1	70.7±11.4
Diabetes Duration (yrs)	8.1±9.9	11.9±9.5	11.4±9.9	14.7±13.7
HbA1c (%)	7.4±1.5	7.7±1.5	7.7±1.5	7.9±1.4
microangiopathy n° (%)	80 (20)	71 (35)	74 (31)	46 (52)
macroangiopathy n° (%)	89 (23)	59 (29)	79 (33)	48 (54)

Conclusion: This study showed a high prevalence of diabetics at risk of ulcer and underlined the importance of an accurate screening in all diabetics at least once. The subsequent classification of the patients in different classes of risk is an important step in order to strengthen both educational and therapeutical strategy in those patients at higher risk of developing ulcers. Our data showed that many patients are not at glycaemic target and underlined the necessity to intensify treatment both in low risk classes, in order to really prevent the development of complications, and in high risk classes, in order to reduce the progression of the complications already present.

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Characteristics of diabetic charcot foot in Western Pacific region - ASIPAC foot study 2
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Aims: The aim of this study (ASIPAC FOOT STUDY-2) was to investigate the characteristics of diabetic patients with Charcot foot in 6 tertiary care hospitals in 6 countries (Thailand, Indonesia, China, Philippines, Vietnam and Japan), and to increase awareness of diabetic Charcot foot problems in Western Pacific Region (WPR).
Methods: The study population includes 54 patients presenting with Charcot foot. Data on patient characteristics, as well as foot characteristics were obtained.
Results: General patient characteristics were different in some points between countries. The patients at the onset were in their fifties in most countries except China (average age (years): 50.0 in Philippines (P), 50.7 in Japan (J), 52.9 in Thailand (T), 53.3 in Indonesia (I), 56 in Vietnam, and 65.2 in China (C)). Body mass index (BMI) was lower than 26.0 kg/mm^2 in most countries (mean BMI: 22.4 in V, 25.0 in J, 25.9 in P, 25.9 in C 25.8 in I and 28.4 in T). Most patients have past history of foot ulcer and amputation before the onset (ulcer (%) / amputation (%): 100/42.9 in C, 100/28.6 in I, 61.5/23.1 in T, 50/5 in J, 40/0 in P, 0/0 in V). Many patients in hot countries such as Philippines, Thailand, Indonesia and Vietnam walked with sandals before the onset. It took a long time until the diagnosis of Charcot foot after the onset (average months: 10.9 in T, 5.7 in J, 4.7 in C, 3.1 in I, 2.2 in P and 0.5 in V). About 50% were diagnosed after presenting with mid-foot deformity together with ulcer (at diagnosis, stage 0 (prodromal period): 1.9%, stage 1 (development): 41.5%, stage 2 (coalescence): 5.7%, stage 3 (reconstruction): 50.9% / location (%) : forefoot: 15.8, mid-foot: 59.7, hindfoot: 24.6 / prevalence of ulcer (%) : 71.4 in C, I, and T, 30 in J, and 20 in P). Even after the initiation of the treatment of Charcot foot, many patients showed the progression of foot deformity, chronic foot ulcer and were undertaken amputation (progression of deformity (%): 42.9 in I, and C, 25 in T, and 5 in J / chronic ulcer (%): 90 in T, 66.7 in C, 71.4 in I and 15 in J / amputation (%): 100 in P, 30.8 in T and 14.3 in C). Most patients were not provided with custom-made footwear despite severe foot deformity.
Conclusion: Characteristics of Charcot patients in the WPR differed from those in Western countries in that the BMI was lower, the diagnosis was made later and the overall prognosis was poorer. It is very likely that there are many

more cases of Charcot foot that are misdiagnosed or undetected in the WPR. In order to improve prognosis and reduce amputations in the WPR, it is essential to have a high index of suspicion for Charcot foot, increase awareness of it and to encourage early presentation of the patient.
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Brachial-ankle pulse wave velocity predicts healing of diabetic foot ulcers among patients with type 2 diabetes
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Background and aims: Some 7-10% of diabetic patients develop chronic foot ulcers, a severe and expensive complication that leads to minor or major amputation in 10-15% of the patients. Peripheral arterial occlusive disease contributes to the impaired healing process. Measurement of pulse wave velocity (PWV) is a useful non-invasive index of arterial distensibility, and predicts cardiovascular morbidity and mortality. Several studies demonstrated that pulse wave velocity a sensitive predicator for peripheral artery disease and cerebrovascular disease among diabetic patients. The objective of this study was to determine whether brachial-ankle pulse wave velocity (baPWV) can predict healing of diabetic foot ulcers.
Materials and methods: We recruited a total of 86 type 2 diabetic patients (52 men and 34 women) with chronic foot ulcers. The age was 62.7±12.3 years, and the diabetes duration was 15.6±11.2 years. Anthropometric, clinical, and laboratory data were measured. All patients were seen weekly for debridement, offloading, and other treatments during the initial 8 weeks. The PWV was measured between the brachial and ankle regions (baPWV), and the baPWV was measured in all patients using a waveform analyser.
Results: At 8 weeks, 31 of the 86 ulcers had completely healed. The 86 patients were divided into two groups according to the clinical outcome of ulcer healing at 8 weeks. There were no differences in age, duration of diabetes, HbA_{1c}, or initial size of the ulcer between the healed and unhealed groups. The healing time of foot ulcers in healed group was 5.2±2.3 weeks (range 1.2-7.7). The baPWV was significantly ($P<0.05$) higher in the unhealed group (1768±290 cm/s) as compared with the healed group (1553±312 cm/s). Age ($r = 0.504$; $p< 0.01$), duration of diabetes ($r = 0.279$; $p< 0.05$), ankle-brachial index (ABI) ($r = -0.328$; $p< 0.05$) and toe-brachial index (TBI) ($r = -0.281$; $p< 0.05$) were significantly correlated with baPWV. But BMI, lipid profiles, HbA_{1c}, and systolic and diastolic BP were not correlated with baPWV. Univariate analysis revealed that baPWV was significantly correlated with healing rate of diabetic ulcers ($r = 0.283$, $p < 0.05$) and age ($r = 0.452$, $p < 0.01$).
Conclusion: We report that increased baPWV is closely associated with the healing time of diabetic ulcers. Our results suggest that the degree of systemic arterial stiffness is predictor of the healing of diabetic ulcers in patients with type 2 diabetes.

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Does the type of off-loading have any impact on the quality of life in patients treated for the diabetic foot?
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Background: We often meet in patients with the diabetic foot ulcers (DFU) with invalidisation, higher morbidity and mortality that could lead to deterioration of quality of life (QoL) and enhancement of depression scale. However, there exist extensive inter-individual psychological differences even in patients with DFU that could be influenced by variety of external factors including the method of DFU off-loading which affects patient's mobility, social relations, employment possibilities, etc.
Aims: In our study we assessed the differences in QoL and depression scale in relation to the type of DFU off-loading and DFU duration between patients with DFU treated by half shoes, orthoses and wheelchairs.
Methods: In total, 48 patients with chronic DFU (mean age 60.6±9.4 years, 77% of males, 80% of patients with Type 2 diabetes, mean diabetes duration 20.5±9.6 years, mean DFU duration 16.5±18.7 months) treated in our outpatient foot clinic from 1/2010 to 3/2010 were consecutively included into our study. Patients were divided into 3 study groups according to the type of using off-loading device - patients treated by half shoes (HS group - 26), by different types of orthoses including TCC (O group - 10) and by wheelchairs (WCh group - 12). QoL was evaluated by standardize questionnaire WHO-QoL-Bref assessing 4 domains (physical capacity, psychological well being, social relationships and environment). Depression scale was assessed by Gender Depression Scale (GDS).
Results: The WCh group did not differ significantly in the depression scale when compared to the HS and O groups (mild form of depression was presented in 85.7% vs. 82.6% vs. 100% of patients; NS; no severe form of depression was found in all study subjects). Moreover, in particular domains of the WHOQoL-Bref, the results of WCh group were similar to the HS and O group (physical capacity-12.5±3.5 vs. 12.3±2.7 vs. 12.7±3.2; psychological well being-14.3±3.1 vs. 14.7±2.7 vs. 15.1±1.6; social relationships-15.3±4.7 vs. 14.7±2.7 vs. 13.5±1.8; environment-14±2.6 vs. 14±2.1 vs. 14.6±1.5; all NS). Study groups differed significantly only in DFU duration (7.3±7.8 in WCh group vs. 14.5±15.9 in HS group vs. 33±25.9 months in O group; $p< 0.01$); other evaluated parameters did not differ significantly between the study groups. Suicidal tendencies and pain symptoms were described in 9.1% (4/44) and 56.1% (23/41) of all questioned patients.
Conclusion: The type of off-loading device including wheelchairs did not influence significantly QoL nor depression scale in patients treated for DFU. We suggest the DFU duration rather than the type of DFU off-loading has a greater impact on QoL and depression in diabetic foot patients.
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The study on mechanisms of epidermal keratinocyte migration impaired by glycated matrix

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Background and aims: Diabetes mellitus is one of the most common disease in human life. Many kinds of complications caused by diabetes metabolism disorder and its metabolite, including nonhealing wound, now is becoming a difficulty in clinical treatment and academic research. Keratinocyte is the mainly repair cell participating wound healing, whose migration function is the base of wound re-epithelization. Keratinocyte continuously migrates on the wound edge, while wound size reduces to complete healing. If keratinocyte migration is blocked, then it means that the wound can't heal. So the study of keratinocyte migration behavior is of profound significance for exploring the rule of wound healing. This study is to discuss that keratinoctye migration is impaired by glycated matrix and its mechanism.
Materials and methods: Keratinocytes from six male Sprague-Dawley rats' back, cultured for two to three generations, were used for experiments. Glycated laminin model were made by laminin cultured in glycolaldehyde and AGEs concentrations were assessed by detecting total fluorescence in glycated laminin model and immunohistochemistry assay. Keratinocytes were cultured on glycated laminin and normal laminin as study group and control group respectively. Keratinocyte migration was measured by scratch wound healing assay. Adhesion rate was expressed by Optical Density(OD), determined with MTT assay. Keratinocyte morphous was observed by scanning electron microscopy and inverted microscope. F-actin was observed by immunofluorescence. Integrinα3 was determined by flow cytometry.
Results: The amount of migrating keratinocyte in study group is significantly less than control (13±4/HP vs 61±11/HP, P<0.05), which confirmed that keratinocyte migration was obviously inhibited by glycated matrix. There was no difference of adhesion rate between study group and control group(12h OD: 0.102±0.014 vs 0.134±0.062; 24h OD: 0.181±0.050 vs 0.187±0.061,P>0.05), however the morphous of keratinocyte on glycated laminin indicates that the cell body was small and hardly spread compared with that on normal laminin. Microfilament of the keratinocyte on the glycated laminin was sparsely distributed in the cytoplasm and around cell nuclear, but the expression of microfilament in the control group was intensively and distributed on the cell membrane, especially on the free edge. The expression of keratinocyte integrinα3 on normal laminin is significantly higher than that on glycated laminin(11.23±5.27% vs 36.58±11.24%, P<0.05).
Conclusion: Keratinocyte migration is inhibited by the glycated laminin. The reason behind the phenomenon is possibly that integrin signaling disorder leads to decrease of integrin and actin expression, followed by the drop of lamellipodia and filopodia development, and the ultimate consequence would be the contribution to the restrained migration.
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IGF-1 is a mediator of inflammation in Charcot Neuroarthropathy and could play an important role in its pathogenesis

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Background and aims: Charcot neuroarthropathy (CN) is a multifactorial disease in which genetics of the axis RANK-RANK-L- osteoprotegerin and a dysregulation of inflammation seem to be involved. A simultaneous improvement of bone mass density of the foot and a reduction of IGF-1 levels was showed after a treatment with alendronate. Aim of this study was to investigate the possible role of IGF-1 in the modulation of inflammation in CN and further support the functional involvement of the axis RANK-RANK-L-osteoprotegerin (OPG) in its pathogenesis.
Materials and methods: Monocytes were obtained from peripheral blood of 10 healthy Donors (C), 10 subjects with CN not in acute phase and 10 subjects with diabetic neuropathy (DN) but without CN. They were incubated with LPS, that is an inflammatory stimulus and in vitro inhibits RANK rna expression, or IGF-1 and then was measured the production of RANK (expressed as percentage variation compared to C) and prostaglandin E2(PGE-2) levels (RIA method, pg/ml) as marker of inflammatory activation.
Results: At baseline there were no differences about Rank expression and PGE-2 levels among CN, ND and C. After incubation with LPS, CN showed a significant smaller reduction of RANK compared to ND and C, while there were not significant differences about PGE-2 levels, although they were slightly higher in CN (402.2 ± 35.64 vs 353.2 ± 40.88 in C and 381.6 ± 54.87 in ND). After incubation with IGF-1 there were not significant differences about RANK expression among three groups while CN showed a significant increase of PGE-2 levels (144.9 ± 30.93) if compared to DN (46.33 ± 12.24) and C (28.89 ± 6.24) p<0.01.
Conclusion: This study seems to support for the first time the possible involvement of IGF-1 as mediator of inflammation, demonstrated by the increased production of PGE-2 after its stimulus, in the pathogenesis of CN, and further confirm the pivotal role played by the axis RANK-RANK-l-OPG.

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Wound healing is selectively modulated by estrogen receptors in diabetes

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Background and aims: Impaired wound healing in diabetes is a major medical and economical problem. It is therefore a need to find new therapeutical approaches. The effects of estrogen on cutaneous wound healing are well established and it might explain the defective wound healing in elderly. Estrogen receptors beta (ERβ) have been linked to venous ulcers. However, the effect on diabetic wounds is still unexplored. The present study analyzed the contribution of the Estrogen receptors (ERα and ERβ) to wound healing in diabetic mice.
Materials and methods: We studied the effect of streptozotocin induced diabetes on wound healing rate in estrogen receptor knock out (ERα-ERKO & ERβ-BERKO) and in wild type mice (C57BL/6). The wound model consists of full-thickness wounds made on the dorsum of the animals. The wound area were determined every second day using a digital camera. Wound granulation, dermal and epidermal regeneration were evaluated by hematoxylin and eosin staining and angiogenesis by GS-1 isolectin staining. Markers for inflammation, endothelial precursor´s cell recruitment and cell migration were analyzed by qRT-PCR. Invitro cell migration assay was carried out in Human dermal fibroblasts (HDFs) in order to determine rate of migration in presence of agonists for estrogen receptors alpha and beta.
Results: Diabetic BERKO mice but not diabetic ERKO mice have a faster wound healing rate compared to diabetic wild type mice (50% wound closure at 3.4 +/- 0.3 days (p<0.05), 4.5 +/- 0.5 days respectively 4.7 +/- 0.5 days). HDFs treated with either specific alpha or beta estrogen receptor agonists showed a significant increase in migration rate.
Conclusion: After induction of diabetes β-receptor knock-out (BERKO) mice display an accelerated wound healing rate when compared to α-receptor knock-out (ERKO) or wild type mice (C57BL/6). These data suggest the use of specific ER agonists for therapeutic trials. The different effect of the ERs on wound healing rate is not due to a specific effect on fibroblast migration rate.
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Emotional distress may impede diabetic foot ulcer healing through elevated levels of interleukin-6: preliminary findings

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Background and aims: Emotional distress induced up-regulation of Interleukin-6 (IL-6) has been found to be detrimental to health. As diabetic foot ulcers are characterized by chronic inflammation, it is plausible that emo-

tional distress could delay the healing of such ulcers by up-regulating and sustaining high levels of pro-inflammatory cytokines such as IL-6.
Materials and methods: Forty five type 2 DM patients (76% male; mean age 55yrs) with plantar neuropathic diabetic foot ulcers (University of Texas Classification: 82% grade 1A; 12% 1B; 3% 2A; and 3% 2B) completed at baseline the self-report measures both of generalized emotional distress (Perceived Stress Scale, PSS: 1.3±0.8 and Hospital Anxiety and Depression Scales: HADS-Anxiety: 13.5±4.4; HADS-Depression: 11.7±5.0) and foot ulcer-specific emotional responses (Neuropathy and Foot Ulcer-Specific Quality of Life, NeuroQoL-Interpersonal Burden scale: 3.7±1.2 and Patient Interpretation of Neuropathy, PIN, scales: PIN-Amputation Worry: 4.1±0.8; PIN-Anger at Practitioners: 2.3±1.0). Ulcer-specific IL-6 levels were determined via quantification of immunohistochemical wound biopsy tissue localization; systemic IL-6 was measured from patient serum via enzyme-linked immunosorbent assay.
Results: In the bivariate analyses, more severe generalized and foot-ulcer-specific emotional distress was associated with higher baseline levels of ulcer biopsy log-transformed IL-6 (raw mean: 608.3±545.5 cells/mm2): HADS-Anxiety (r=0.44; p=0.031), PSS (r=0.44; p=0.034), PIN-Amputation Worry (r=0.54; p=0.007) and NeuroQoL-Interpersonal Burden (r=0.60; p=0.004). Similarly, more severe PSS (r=0.35; p=0.05), PIN-Anger at Practitioners (r=0.33; p=0.03) and NeuroQoL-Interpersonal Burden (r=0.39; p=0.03) were associated with higher baseline serum IL-6 levels (mean: 8.9±4.2pg/ml). Furthermore, patients with higher baseline levels of serum IL-6 were less likely to achieve 80% or greater wound area reduction at 6 weeks (r=-0.35; p=0.03).
Conclusion: These preliminary findings suggest that emotional distress-related IL-6 up-regulation may be one of the mechanisms linking psychological stress to chronicity of diabetic foot ulcers.
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Characterisation of circulating stem and progenitor cells in type 2 diabetic patients with foot ulceration
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Background and aims: Type 2 diabetes (T2DM) is a major health problem in Europe due to severe complications, for example the diabetic foot syndrome (DFS). Ulcerations that are often difficult to heal. Their pathogenesis may be associated not only with metabolic abnormalities but also with alterations in stem and progenitor cell mobilization. It has been shown that both the functionality and the number of endothelial progenitor cells (EPC) circulating in peripheral blood (PB) are altered in diabetic patients. However, other stem cell (SC) populations potentially involved in wound healing and regeneration such as i) mesenchymal SC (MSC) and ii) adult pluripotent SC (PSC), including very small embryonic-like (VSEL) SC, have never been examined in T2DM. Aim of the study was to examine the phenotype and level of SC circulating in PB in T2DM subjects with and without DFS as compared to healthy controls.
Materials and methods: Three groups of subjects were included: a) T2DM patients without DFS (n=9); b) T2DM with DFS with ulceration(s) (n=3), c) controls without diabetes (n=4). We employed flow cytometry to evaluate the presence of the following SC populations based on the surface markers: i) EPC (CD31+CD133+CD45-, CD31+KDR+CD34+CD45-); ii) MSC (STRO-1+CD105+CD45-, CD90+CD29+CD45-) and PSC (Lin-CD45-CD133+, Lin-CD45-Tra1.81+).
Results: We found that when compared to healthy subjects, the number of EPC was decreased in both T2DM groups (w/o and with DFS) (9.6±2.1, 5.3±0.7* and 3.4±2.3 of CD31+CD133+CD45- cells/1ml PB, respectively). Similarly, the number of MSC were smaller in both T2DM groups (6.4±1.1, 3.9±0.5* and 2.2±0.4 of STRO-1+CD105+CD45- cells/ 1ml PB, respectively). Interestingly, the numbers of PSC was slightly decreased in healthy controls as compared to T2DM patients (0,27±0.7, 0.30±0.08 and 0.38±0.07of Lin-CD45-CD133+ cells/ 1ml PB, respectively). * P<0.05 vs. control.
Conclusion: For the first time we characterized several subsets of SC circulating in the blood of T2DM patients. Our initial results indicate that as compared to healthy controls, their mobilization may be different, particularly in the subgroup with DFS and ulceration; a potential pathogenic role of this phenomenon should be considered.
Supported by: European Union structural funds, Innovative Economy Operational Programme

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The diabetic foot: relevance of endothelial progenitor cells as a prognostic marker of mortality and disease progression
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Background and aims: Ischemic diabetic foot ulcers represent an unmet clinical need. To date, the prediction of clinical outcome relies on clinical data rather than on endogenous repair mechanisms. Circulating endothelial progenitor cells (EPC) are implicated in healing processes but reduced in patients with diabetes and inversely correlated with severity of vascular complications. Here, we report the preliminary results of a longitudinal study aimed to verify whether the abundance and functional activity of EPCs predict major endpoints such as amputation and post-angioplasty restenosis.
Materials and methods: The project was designed to enrol 109 diabetic (type 1 and 2) patients and 30 age- and sex-matched non-diabetic subjects referring to our Institution for chronic critical ischemia as defined by the guidelines of the Inter-Society Consensus (TASC) for the management of peripheral artery disease. Baseline testing includes measurement of glycemia, HbA1c, echo-doppler, transcutaneous oximetry (TO) and angiography. A blood sample (30mL) is obtained for isolation of mononuclear cells (MNCs). The migratory activity of isolated MNCs is measured in a transwell migration assay using SDF-1α (100ng/ml) as a stimulus. The antigenic profile of freshly isolated and migrated/non migrated MNCs is evaluated by flow cytometry. All patients are subjected to percutaneous angioplasty and follow up visits are scheduled at 1, 3, 6, 12 and 18 months.
Results: Thirty-four diabetic patients and seven non-diabetic subjects were enrolled to date with an average follow up of 7 months. No fatal event was recorded; 1 major amputation and 5 restenosis occurred in the diabetic group. The number of MNCs did not differ between the 2 groups. EPCs (CD34+KDR+CXCR4+CD45low) tended to be reduced in diabetic (0.009±0.002% vs. 0.012±0.003% in non-diabetic p=0.4). In non-diabetics, SDF-1α stimulation resulted in a 2-fold enrichment of EPCs in the migrated fraction, whereas the response of diabetic EPCs to SDF-1α was totally abrogated. With regard to clinical endpoints, complications were associated with a higher migratory activity of lineage-positive subpopulations of the MNC pool, whereas no difference was found in the number and migratory activity of EPCs. Ad interim analysis of independent variables (number and migratory activity) indicates that the designed study is adequately powered to reach definite conclusions on the predictive value on major endpoints.
Conclusion: This is the first longitudinal study assessing the predictive value of circulating progenitor cells in patients with foot ulcers and critical limb ischemia. Results indicate that diabetes impairs the migratory deficit of circulating progenitors and that unbalanced migratory activity of different MNC subfractions may be associated to a higher risk for complications in the diabetic cohort. Our preliminary data also indicate that the study has enough power to determine whether circulating progenitor cells may represent a valuable biomarker of vascular events in patients with foot ulcers undergoing revascularization.
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Explanations for lower peak plantar foot pressures in Indian Asians versus Europeans with type 2 diabetes
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Background and aims: Risk of diabetes-related foot ulceration and amputation is substantially lower in Indian Asians versus White Europeans in the UK. We have also recently demonstrated that neuropathy (large and small fibre) is less prevalent in Asians, probably accounting for much of the reduced Asian ulcer risk. We now aimed to: (i) compare peak plantar foot pressures, an established risk factor for ulceration, in Indian Asian and European diabetic subjects; (ii) explain any ethnic pressure differences found.
Materials and methods: From our cross-sectional study of a population-based sample of age- and sex-matched adults with type 2 diabetes of European and Asian descent in the UK, a random sub-cohort of 104 Europeans (50 female: 54 male) and 105 Indian Asians (36 female: 69 male) underwent plantar foot pressure measurements using the semi-quantitative PressureStat® system. Ethnic differences in peak pressures were determined at the meta-

tarsal heads, great toe, second toe and heel. Comparisons with a group of 34 age-matched, non-diabetic control subjects (European (12 female: 5 male) and Asian (7 female: 10 male)) were also sought.
Results: Peak pressures (>6kg/cm^2) at metatarsal heads 1, 2, 3, and 5 were consistently more prevalent in the European vs. Asian diabetic group ($p<0.01$). Furthermore, 32% Europeans had ≥4 peak pressure sites compared with just 6% Asians ($p<0.0001$). Logistical regression analysis revealed that fewer peak pressures in Asians vs. Europeans could be partly explained by Asians' lower weight, less plantar callus and more vigilant footcare (attenuating the Odds Ratio from 0.13 (0.05-0.32, 95% CI; $p<0.0001$) to 0.28 (0.10-0.78; $p=0.014$)), however most of this ethnic difference remained unaccounted for. Asian and European control groups showed a consistent trend for fewer peak pressures at plantar sites than their diabetic counterparts, yet peak pressures at the great toe were, conversely, greater. PressureStat® demonstrated a high degree of intra-user agreement with 97.4-99.0% of scores within one range of each other on re-scoring.
Conclusion: Type 2 diabetic Indian Asians have significantly lower peak plantar pressures than their European counterparts, which can, in part, be attributed to lower weight and less plantar callus in Asians. These data may further help explain the substantially reduced Asian foot ulcer risk.
Supported by: Diabetes UK

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Methicillin resistant staphylococcus aureus in diabetic foot ulcers of a Chinese care hospital: risk factors for infection and prevalence
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Background and aims: Retrospective case-control study of 118 (M: F, 68:50) Chinese type 2 diabetic patients with foot ulcers (Wagner's grade 3-5) were studied to determine the prevalence and risk factors for methicillin-resistant Staphylococcus aureus (MRSA) infection, in relation to community or hospital original parameters.
Materials and methods: Ulcer specimens were processed for smear for Gram's staining, aerobic culture, and susceptibility identifications. Staphylococcus species were tested for methicillin resistance by using oxacillin.
Results: S.aureus was the most frequent pathogen (25.6%) in this population, a high proportion of S.aureus isolates were MRSA (63.4%). 65.4% met the definition of hospital associated MRSA (HA-MRSA) infections. Size of ulcer (adjusted OR 1.61; 95% CI 1.22-2.12) and osteomyelitis (adjusted OR 18.51, 95% CI 2.50-137.21) were independent predictors of MRSA infection. The HA-MRSA group had significantly different distributions from the community associated MRSA (CA-MRSA) group with respect to age, long history of diabetes, and length of hospital stay (all P<.001). Neuropathy, vascular disease (all P= .049), and osteomyelitis (P= .026) were the most common underlying conditions observed in the HA-MRSA group.
Conclusion: This study makes contribution to precaution against the emergence of MRSA including different acquired MRSA among the Chinese population with diabetic foot ulcers based on their original or clinical parameter.
Supported by: National Nature Science Foundation of China

1163

The influence of contrast medium on renal function in diabetic patient with critical limb ischaemia after contrast angiography/peripherial transluminal angioplasty
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Background and aims: The aim of the study was to assess the renal function of diabetic patient with critical limb ischemia (CLI), who underwent contrast angiography (CA)/peripherial transluminal angioplasty (PTA) before and after administration contrast medium (CM).
Materials and methods: 82 patients with PAD recruited in the study (mean age 65±17 years; male/female 47/53%; Type 2 DM 92%; mean diabetes mellitus (DM) duration 17±10 years). Peripherial artery disease was assessed by palpation of pedal pulse, ankle brachial index (ABI) measurement, doppler, transcutaneous oximetry (TcPO2) and duplex scanning (DS). Patients were divided according frequency and volume of CM and accuracy of DS results into 3 groups. The groups were matched by type of DM, age, duration of DM, sex. Group A (n=28) were underwent CA with the mean volume of CM 200 ml and consecutively PTA with the mean volume CM 130 ml. In group B (n=36) endovascular revascularizations were performed with the mean volume CM 130 ml without CA, because of highly valuable of DS results. In group C (n=18) multiple CA and PTA were done with the mean volume CM 480 ml summary. Glomerular filtration rate (GFR calculated by MDRD equation) assessed before and after administration CM on 3-5 days and in a 14 months in all groups.
Results: Initial albumin excretion rate (AER) and GFR were not different in comparing groups. The decrease of mean GFR was 20,2±2,1 ml/min/1.73m^2 in group A; 14,3±1,7 ml/min/1.73m^2 in group B; 19,1±1,9 ml/min/1.73m^2 in group C on 3-5 days after administration of CM. $p_{A-B}<0.01$, $p_{A-C}<0.2$, $p_{B-C}<0.01$. The decrease of mean GFR was 22,2±1,6 ml/min/1.73m^2 in group A; 15±1,6 ml/min/1.73m^2 in group B; 24±1,7 ml/min/1.73m^2 in group C in a 14 months. $p_{A-B}<0.01$, $p_{A-C}<0.2$, $p_{B-C}<0.01$. 1 case of contrast-induced acute renal failure was registered in group A. Microalbuminuria progression according AER was observed in 3 cases in group A and in 2 cases in group C. Neither AER progression nor renal failure were documented in group B. The accuracy and sensitivity of duplex scanning was comparable in the selection of aorto-iliac lesions (86% and 88%) and femoro-popliteal lesions (91% and 93%) in A and B groups, but in infrapopliteal axis these characteristics were significantly higher in group B versus group A, 90% and 78%, respectively.
Conclusion: The results of this study showed that the risk of progression diabetic nephropathy in patients with CLI after CA/PTA increased and depend on frequency and volume of CM. The high accuracy DS in diabetic patients with CLI permits to reduce the frequency of preliminary angiography and prevent the development of deteriorating of renal function.

PS 114 Diabetic foot - treatment

1164

Podiatric insoles cause foot ulcers in diabetic patients

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Background and aims: Inadequate footwear is an important contributor of foot ulceration in diabetic patients with polyneuropathy and prescription of protective footwear is seen as a cornerstone in preventing ulcers. In several countries preventive diabetic foot care is provided by podiatrists, who can also prescribe insoles. However, recent publications have casted doubt about the effectiveness of these insoles to reduce plantar pressure. Therefore we studied the effect of podiatric insoles on the incidence of diabetic foot ulcers.
Materials and methods: This study was part of the Podopoof trial; in this RCT we compared podiatric (n=284) with usual care by diabetologists (n=285), in diabetic patients with neuropathy and moderate risk (category 2 IWDGF) for ulceration. Podiatric care consisted of ≥ 2 consultations/year and included preventive foot care as well as prescription of podiatric insoles to reduce plantar pressure, if deemed necessary. New cases of diabetic foot ulcers were ascertained during a follow-up period of up to 3 years.
Results: As reported earlier no difference were observed in ulcer incidence between the podiatry and usual care groups, 28 vs 30; data of the intervention and control group were therefore combined. The mean age was 63 years, the modified neuropathy disability score was 3,1, 52% were male. Insoles were prescribed in 184 patients, 177 (62%) in the podiatry and 7 (2%) in the usual care group. Of the patients with an ulcer 65% had insoles. Of the patients with insoles 22 (12%) developed an ulcer, while this occurred in 6 (1%) without insoles (p<0.01). Multivariable Cox regression analysis showed that the time to ulceration in the insole-group is shorter than in the non-insole group. Subgroup analysis showed that men develop more ulcers than women and that the detrimental effect of insoles was also larger in men.
Conclusion: Prescribing preventive insoles for diabetic patients with moderate risk for ulceration with the goal to prevent ulceration can have opposite effects: in the insole-group more ulcers occurred than in the non-insole group. It is our belief that podiatrists should not prescribe insoles in these patients to prevent ulceration.
Supported by: CVZ, Netherlands

1165

Rate of relapses diabetic osteoarthropathy and its dependence on the terms and a carrying mode of weight-bearing bandage TotalContactCast at patients with acute Charcot foot

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Background and aims: The aim was to estimate the results of the treatment Charcot foot (Ch.f) in type 1 and 2 diabetic patients with weight- bearing by TotalContactCast at acute stage of Ch.f.
Materials and methods: In total 47 patients with diabetes mellitus (DM) 1 type (n = 21) and 2 types (n=26), mean age of patients - 49,7 ±13,8 years. All patients divided in 2 groups by temperature criteria: group 1 at 22 patients (Δtº- 5,7º±2,7), and Group 2 - at 25 (Δtº-4,2±2,1). Mean of HbAlc at patients in Group 1 was 9,3 ± 2,34 %, in Group 2 - 8,7 ± 1,6. Difference between occurrence of clinical symptoms diabetic osteoarthropathy and start of treatment was 10,5 ±9 month at patients in group 1, 19,7±15,9 years at patients in group 2. To all patients duration with treatment by TotalContactCast of group 1 was 4,9 ± 1,8 month, in group 2 -7,7 ± 3,6 month. To all patients' correction of therapy diabetes mellitus (DM) metabolic control has done. Differences between groups were examined by using Fisher criteria, significance level is p<0,05. Duration of follow-up period was 24 ± 2.4 months.
Results: In 24 months the decrease of Δ HbAlc achieved at patients in group 1 - 7,2 ± 1,53%, and 6,9 ± 1,9% in group 2. From 47 patients, 68 %(n=32) had good compliance of caring TotalContactCast and at 28% (n=9) relapse osteoarthropathy was observed. 31% of patients (n=15) didn't follow the term of recommended treatment and the relapse of osteoarthropathy was significantly high and observed in 67 % cases (n = 10); remission in 23%, (p = 0,023). In group 1- 22 patients only 63 % (n =14) followed recommendations about carrying period of TotalContacCast and unloading, thus relapse in group 1 observed at 43 % (n=6). Significantly associated in 36 % (n=8) patients, who stopped therapy before term, relapse was revealed at 62 % of patients (n=5), (p = 0,008). In group 2 of 25 patients - of 72 % (n=18) followed the term of carrying TotalContactCast and lower extremity unloading (relapse - at 17 % of patients, n=3). From 28 % (n=7) the patients without observing a mode of unloading, in 71 % of cases (n=5) relapse osteoarthropathy (p = 0,037) is revealed.
Conclusion: Weight-bearing bandage is a highly effective method of treatment of the first choice for therapy Charcot foot: remission of the complication is reached in 72 % of cases, in both groups. At non-observance of conditions fixating therapies remission in 2 year evaluating period makes only 23 % of investigated patients. Thus, the mode of weight-bearing and its duration is crucial to lower the relapse rate of diabetic patients with acute osteoarthropathy, within glycemic control.

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Diabetic foot osteomyelitis can be successfully treated with antibiotics

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Background: Osteomyelitis (OM), a common complication of diabetic foot ulcer, is associated with higher risk of amputation. In our centre we treat OM primarily with antibiotics and we wanted to study the outcome of patient who had diagnosis of OM.
Aims of study: The aim of this study was to analyse clinical outcome of subjects who had diagnosis of OM in the past 5 years.
Subjects and methods: In this retrospective study, cases were selected from the electronic record with the diagnosis of OM. Results were crosschecked with radiology database. Pathology and microbiology database were also used to collect data.
Results: 147 cases had clinical diagnosis of OM out of which 130 (mean age 66.2±14.4 years and mean duration of diabetes 13.2±10.9 years) had diagnosis reconfirmed on at least one of the established criteria (Probe to bone 102, X-Ray changes 69, Bone scan 27, leukoscan 4 and bone biopsy 5). Of these reconfirmed cases, majority (66.9%) were male and had type 2 diabetes (80%) with mean HbA1c of 8.1± 2.1% and cholesterol of 4.2±1.5 mmol/L. Peripheral vascular disease, defined by absence of palpable pulses, was present in 61 (46.9%) subjects. Blood count performed on 112 cases showed raised neutrophil count (>7.5) only in 26 (23.2%). 64 had staphylococcus isolated from wound swab of which 20 (31.3%) had MRSA. Flucloxacillin and fusidate combination was used in 81 cases whereas ciprofloxacin and clindamycin combination was used only in 17 cases. 87 (66.9%) healed with single (n=46) or multiple (n=41) courses of antibiotics. 18 (13.8%) had amputation of which 16 (12.3%) were minor (Toes or Ray amputation) and 2 (1.5%) were major (above or below knee). 12 (9.2%) had vascular intervention (8 angioplasty & 4 bypass) and 8 (6.2%) died within 12 months of diagnosis due to other causes. There were no differences in outcome between subjects with or without x-ray changes. When compared between those which healed (n=87) and those patients who died or needed amputation (n=26), there was no difference in age sex, duration of diabetes, site of ulcer, presence of x-ray changes or peripheral vascular disease. OM due to MRSA was the only factor that predicted adverse outcome (21.1% vs 53.3%; p=0.04). Higher rate (p=0.01) of adverse outcome was noted in patients using combination of ciprofloxacin and clindamycin, which may be due to its use as a last resort in our clinic.
Discussion: Our data confirms that OM can be successfully treated with antibiotics. Flucloxacillin and fusidate can be used as first line treatment in majority of cases. Surgery should be reserved only for cases that fail to respond to medical treatment.

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Efficacy of moxifloxacin in the treatment of diabetic foot infections: results of the RELIEF study

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Objectives: Diabetic foot infections (DFIs) cause substantial morbidity. As DFIs are usually polymicrobial, broad-spectrum antibiotics play an important

role in therapy. Due to their wide antimicrobial coverage and pharmacodynamic properties, fluoroquinolones, such as moxifloxacin (MXF), may have several advantages over other antimicrobial classes. The RELIEF study was conducted to provide further data on the efficacy of MXF in specific complicated skin and skin structure infections. Data on DFIs are presented here.
Methods: In this double-dummy, double-blind, randomised, controlled trial, patients with a DFI requiring antimicrobial therapy were stratified according to infection severity and the requirement for surgery. Patients received either IV/PO MXF 400 mg qd or IV piperacillin/tazobactam 4.0/0.5 g tds followed by PO amoxicillin/clavulanate 875/125 mg bd (PIP/TAZ-AMC), for 7-21 days. The DFI diagnosis was based on predetermined criteria, documented by repeated photographs and confirmed by an independent data review committee (DRC). The primary efficacy variable was resolution of infection 14-28 days after completion of study medication (test-of-cure, TOC) as determined by the DRC.
Results: A total of 206 patients (mean age 59.2 years) were valid for the efficacy analysis (MXF=110, PIP/TAZ-AMC=96). Of these, 65.5% of MXF- and 70.8% of PIP/TAZ-AMC-treated patients had clinical signs of peripheral arterial disease (ankle brachial index <0.9, foot pulses barely or non-palpable). Most patients had DFIs with a PEDIS score of 3, i.e. moderate in severity (MXF 81.3%; PIP/TAZ-AMC 86.2%). In the microbiologically-valid (MBV) population, polymicrobial infections were common (MXF: 60.9%; PIP/TAZ-AMC: 62.4%); the most frequently isolated pathogen was *S. aureus* (MXF 69.6%; PIP/TAZ-AMC 81.2%). Meticillin-resistant *S. aureus* was relatively uncommon in the MBV population (MXF 12.0%; PIP/TAZ-AMC 14.1%). A total of 150 (72.8%) patients had initial surgery (MXF 70.9%; PIP/TAZ-AMC 75.0%), including amputation in 46.4% (MXF) and 34.4% (PIP/TAZ-AMC) of PP patients. MXF and PIP/TAZ-AMC had similar efficacy with respect to clinical cure at TOC (Table). A total 20.9% MXF and 25.0% PIP/TAZ-AMC patients had additional surgeries >48 hours after the start of therapy and were assessed as clinical failures. Bacteriological success rates were comparable between treatment arms (Table).
Conclusion: IV/PO MXF had similar efficacy to IV PIP/TAZ-AMC in patients with DFI. MXF can be considered a valuable option for the treatment of moderate-to-severe DFI.

Table: Clinical and bacteriological success rates at TOC overall and by most commonly isolated pathogen

	MXF	PIP/TAZ-AMC	$P^{\ddagger}$ (95% CI)
	n/N (%)		
Clinical cure[§]	84/110 (76.4)	75/96 (78.1)	0.650 (-14.5, 9.0)
Bacteriological success (MBV population)			
Overall	66/92 (71.7)	61/85 (71.8)	0.658 (-16.9, 10.7)
Staphylococcus aureus Meticillin-susceptible Meticillin-resistant	 43/53 (81.1) 8/11 (72.7)	 39/57 (68.4) 10/12 (83.3)	 - -
Escherichia coli	6/8 (75.0)	6/9 (66.7)	-
Enterococcus faecalis	19/30 (63.3)	20/29 (69.0)	-

‡Cochran-Mantel-Haenszel test
§n/N=number of patients experiencing clinical cure/ total number of patients
n/N = number of patients experiencing eradication or presumed eradication/number of patients with pathogen isolated and an evaluable bacteriological response
CI: confidence interval; MBV: microbiologically valid population (all efficacy valid patients with a pathogen isolated at baseline)

Supported by: Bayer Schering Pharma AG

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The effect of topical phenytoin on healing in diabetic foot ulcers: a randomised controlled trial
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Background and aims: Phenytoin (PHT) may have a positive effect on wound healing by increasing collagen production and reducing bacterial load and wound exudate. A randomised, controlled, double-blind, clinical trial was conducted to evaluate the effect of topical PHT on healing in diabetic foot ulcers (DFU).
Materials and methods: A PHT dressing and a control dressing were manufactured. Participants of ≥ 18 years of age with peripheral neuropathy, stable vascular status, and a DFU ≥ 4 weeks duration were included. Participants with renal disease, acute ischaemia, necrosis, worsening infection or osteomyelitis were excluded. Subjects were independently randomised to either PHT or Control groups, received standard wound care, and dressing application. Primary end-point analysis (DFU closed or not at 16 weeks) was calculated by Survival Analysis. Analysis of secondary outcome (percentage change in DFU area over time) used an Ordinal Regression Model.
Results: Participants (n=65, 52 with Type 2 Diabetes) were randomised to the PHT (31) or Control group (34). Following a maximum of 16 weeks treatment, 60% of the DFUs closed overall (18 PHT: 20 Controls) with no statistically significant differences in complete healing or in DFU area over time between the two groups. Pain levels were reduced in the PHT-treated group. At 24 weeks, 1 DFU had recurred.
Conclusion: There were no differences in DFU closure rates or in DFU area over time between the two groups. This study does not support the use of PHT in the treatment of DFUs.
Supported by: HSCR&DNI

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Improved survival in patients with diabetes and chronic foot ulcers after hyperbaric oxygen therapy. Outcome of a randomised double-blind placebo controlled study
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Background and aims: Presence of diabetic chronic foot ulcers (DFU) is associated with an increased mortality risk. Hyperbaric Oxygen Therapy (HBOT) has been suggested as a treatment modality of DFU. HBOT increases oxygenation and stimulates angiogenesis. The aim of this study was to evaluate if HBOT improves survival in patients with diabetes and chronic foot ulcers.
Materials and methods: Hyperbaric Oxygen therapy in patients with Diabetes and chronic Foot Ulcers (HODFU) study is a prospective randomized double-blind placebo-controlled study evaluating the effect of 40 HBOT sessions as compared to 40 treatments with hyperbaric air (placebo). Patients receiving more than 35 treatment sessions were included in the predefined per-protocol analysis. Three-year mortality rates were evaluated in this study. Categorical variables were analyzed using Fischer´s exact test, continuous variables using Mann-Whitney U-test and Kaplan-Meier curves using Cox-Mantel test. A two-sided p-value <0.05 was taken as statistical significant.
Results: 75 patients (38 HBOT and 37 placebo) with a similar median age (67 and 71 years (n.s.) (HBOT and placebo)) and a diabetes duration of 22 and 21 years (n.s.) were included in this analysis. No differences were seen in comorbidity between groups. Mortality rates were 10.5 % and 29.7 % (p=0.04) respectively after three years follow-up. Median ages of deceased patients were 79 and 75 years (n.s.).
Conclusion: This study indicates that HBOT may improve survival in patients with diabetes and chronic foot ulcers.
Supported by: Mrs Thelma Zoegas Foundation

1170

Pathogenetic criteria of differentiation tactics at surgical treatment of purulent necrotic wounds in patients with diabetic foot
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Background: A search for informative criteria for a wound process course in the treatment of destructive forms of Diabetic Foot Syndrome (DFS) presents a serious clinical problem, which requires new scientific approaches in its solution.
Aims: Development of prognostic criteria for a wound process course allowing choosing pathogenetically justified methods of surgical treatment of wounds in patients with DFS.

Material and methods: The results of a surgical treatment of 186 patients with destructive forms of DFS have been analyzed in accordance with PEDIS classification with $P_{1-2}E_{1-2}D_{2-3}I_{2-3}$ present. The condition of the intracellular tyrosinase system, the content of cytokines (IL-1β, IL-4, TNGα, IFNγ) and eicosanoids (PGE_2, LTB_4, PGI_2) in blood at different stages of the treatment have been studied. A wound coating, autoplasty, a tamponade by dermo-fat flap (DFF) and a culture of cultivated autofibroblasts have been used as means of covering postoperative wound defects, stabilization of a destructive process and acceleration of tissue reparation.
Results: The individual peculiarities of a wound process course were directly dependent on the area and depth of tissue destruction, power of the tyrosinase system, which finds its expression in the results of the tyrosinase index. After a surgical treatment of the wound, changes in the intercytokine coefficient allowed prognosing the course of a proliferation phase - 50% and higher increase of the coefficient was a favorable sign and an indication for the use of a wound coating or autoplasty. 10% decrease of the coefficient and lower appeared to be an unfavorable sign and required the use of a tamponade of a great wound defect by DFF and/or a culture of cultivated autofibroblasts with a further plastic covering of the wound. 14.3% decrease of the eicosanoid coefficient during 3-5 days after a necrosectomy was accompanied by a high efficiency of a wound coating and autoplasty application, quick wound healing. 33.5% and higher increase of the eicosanoid coefficient against the background of LTB_4 and PGI_2 deficiency testified to the humoral control disorder of inflammatory reparative processes which were accompanied by spread of a destructive process, a more prolonged period of wound healing and/or resulted in performing big amputations.
Conclusion: The reparation humoral regulators revealed reflect individual peculiarities of a wound process course and give an opportunity to make a prognosis concerning the outcome of wound healing in patients with DFS operated on.

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Outcome of surgical treatment in diabetic forefoot osteomyelitis
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Background and aims: Osteomyelitis is one of the most frequent infections of the diabetic foot. The treatment of osteomyelitis of the foot in diabetic patients continues to be debated, most experts considered that the standard should be the surgical removal of infected bone. Aim of this study is to determine efficacy and relapse rate of surgical treatment of osteomyelitis.
Materials and methods: We performed a surgical removal of infected bone in 206 consecutives diabetic patients. Forefoot osteomyelitis were confirmed by probe to bone test and radiological signs of osteomyelitis.
Results: Osteomyelitis were localized in 140 patients at phalangeal level (68%), in remaining 66 patients at metatarsal head level: 19 first head (9%), 9 second head (4%), 5 third head (3%), 4 forth head (2%), 29 fifth head (14%). Bone culture was performed in 122 patients, in 118 patients was positive and Staphylococcus aureus was the organism isolated in majority of cultures (42%). Kind of surgical treatment: 152 conservative surgical procedures were performed (74%), in the remaining cases: 23 distal finger amputations , 20 finger amputations, 11 ray amputations. 154 patients (75%) healed, mean healing time was 62±42 days. Causes of healing failure were: 19 patients for ischemic relapse, 21 patients for residual osteomyelitis, 12 patients for other causes. 37 patients (18%) healed with a second surgical procedure. Once healed wound relapse was observed in only 5 patients (3%) in a mean follow up of 12±4 months.
Conclusion: Surgical removal of infected bone in forefoot osteomyelitis seem a safe procedure with an elevated healing rate and large possibility of conservative management. Relapse rate is low when healing is reached.

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Health Technology Assessment (HTA) on the importance of growth factors for the treatment of Diabetic Foot Ulcers (DFU)
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Background: Ulcers as a result of Diabetes mellitus are a serious problem with an enormous impact on the overall global disease burden due to the increasing prevalence of diabetes. Because of long hospital stays, rehabilitation, often required home care and the use of social services diabetic foot complications are costly. Therapy with growth factors could be an effective and innovative add-on to standard wound care. The aim of the HTA on behalf of the German Institute of Medical Documentation and Information DIMDI is to assess the safety and efficacy of growth factors alone or in combination with other technologies in the treatment of DFU including medical, economical, social, ethical and juridical aspects.
Methods: We systematically searched relevant data bases limited to English and German language and publications since 1990. Cost values were adjusted for the price level in 2008 and converted into Euro. Review and assessment of the quality of publications followed methods conforming to widely accepted standards for evidence-based medicine and health economics.
Results: We identified 25 studies (14 randomized controlled trials (RCT), nine cost-effectiveness analyses, two meta-analyses). The RCTs compared an add-on therapy to standard wound care with standard wound care/placebo alone or extracellular wound matrix: 6 studies used becaplermin, two rhEGF, one bFGF, and five studies the metabolically active skin grafts Dermagraft and Apligraf. Study duration ranged from 12 to 20 weeks and the study population comprised between 17 and 382 patients, average 130 patients. Treatment with becaplermin, rhEGF and growth factors secreting skin implants Dermagraft and Apligraf showed in eight out of 13 studies an advantage concerning complete wound closure and the time to complete wound healing with statistically significant differences. Evidence for a benefit of treatment with bFGF could not be found. In four out of the 14 studies the proportion of adverse events was 30 % per study group with no difference between the treatment groups. The methodological quality of the studies was affected by significant deficiencies. Economic evaluations showed becaplermin being cost-effective whereas no obvious statement can be made regarding Dermagraft and Apligraf because of diverging cost bases and incremental cost-effectiveness ratios.
Discussion: Differences in standard wound care are complicating the comparison of study results. Taking into consideration the small to very small sample sizes and other methodological flaws with high potential of bias the validity of the results with regard to effectiveness and cost-effectiveness has to be considered limited. The duration of treatment and follow-up examinations is not long enough to assess sustainability of intervention and surveillance of ulcer recurrences or potential treatment related adverse events like development of malignancy.
Conclusion: There are indications of an advantage for the add-on therapy with growth factors in DFU concerning complete wound closure and the time to complete wound healing. Further more studies of high methodological quality with adequate sample sizes and sufficient follow-up periods are necessary, also investigating patient-relevant parameters like health-related quality of life, acceptance and tolerance of intervention in addition to clinical outcomes.
Supported by: DIMDI

PS 115 Retinopathy - prevalence and mechanisms

1173

Prevalence of diabetic retinopathy at first screening event in a national screening programme in Wales UK: 2005 - 2009

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Background and aims: To report the prevalence of diabetic retinopathy (DR) in subjects with diabetes who attended for their first screening event with the Diabetic Retinopathy Screening Service for Wales (DRSSW) between 2005 and 2009.
Materials and methods: 135,152 subjects with diabetes (55.3% male, 42.6% female, 2.1% not documented) attended for their first screening event. Digital photography (2x 45° field of each eye) was performed following mydriasis with tropicamide 0.5%; retinal grading was based on the UK National Consensus Grading Protocol with the highest (worst) grade for either eye taken as the final grade. Referral to hospital eye services were made for those cases where the level of DR seen was pre-proliferative (PPDR) or proliferative (PDR) with or without the presence of maculopathy or maculopathy only i.e. referable diabetic retinopathy (RDR). The level of DR was considered to be of a sight-threatening level (STDR) if either or both PDR and maculopathy were present.
Results: 88,131 subjects (65.2%) had no DR and 47,021 (34.8 %) (57.5% male, 40.1% female, 1.5% not documented) had evidence of DR. In those subjects with DR at first screen the mean (±SD) age was 63.3 (14.8) years, duration of diabetes was 9.6 (9.0) years; 11.6% were T1DM, 64.9% T2DM and for 23.5% type not recorded. 13.9% of subjects were diet controlled, 42.2% received additional oral hypoglycaemic agents and 23.8% insulin. In 20.1% the treatment modality was not documented. During the five year screening period (Table 1) the prevalence of BDR at first screening remained essentially unchanged at approximately 80% of those patients with any evidence of DR. PDR increased from 0.9 to 2.6% over the 5 year period but maculopathy remained essentially unchanged between 4.4-5.1%. The number of people requiring referral to ophthalmologists increased slightly following the first year but then remained unchanged.
Conclusion: In a national screening programme at first screen 65.2 % of patients had no DR. Of those with DR (34.8 %) the majority had BDR (~80%) which did not require referral to the hospital eye service. In summary only 5.6% of the total population at first screen required further assessment at the hospital eye service.

Table 1 Presence of DR at first screen (2005 - 2009)

	All Years		2005	2006	2007	2008	2009
	N	%	%	%	%	%	%
BDR	36,724	78.1	80.8	76.5	77.6	76.5	76.7
PPDR	2,490	5.3	4.9	5.6	5.6	5.1	5.3
PDR	736	1.6	0.9	1.4	1.7	2.5	2.6
Maculopathy	2,222	4.7	4.4	5.1	4.8	4.6	4.6
$STDR^1$	5,115	3.8	3.1	4.2	4.0	3.7	3.9
$STDR^2$	5,115	10.9	8.5	11.8	11.5	12.5	12.6
RDR^1	7,605	5.6	4.9	6.3	6.0	5.3	5.5
RDR^2	7,605	16.2	13.4	17.4	17.1	17.6	17.9

BDR-Background DR; PPDR-Preproliferative DR; PDR-Proliferative DR; Maculopathy-Exudates within 1 disc diameter of fovea ±retinal thickening; $STDR^1$-Sight threatening DR of the total population; $STDR^2$-Sight threatening DR of those with DR; RDR^1-Referable DR of the total population; RDR^2 - Referable DR of those with DR.

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Prevalence and associated risk indicators of retinopathy in rural Bangladeshi population with and without diabetes

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Background and aims: Retinopathy, a potential sight threatening condition, is a significant public health problem. The absence of reliable population based epidemiological data on retinopathy in Bangladesh is a serious impediment to the effective national planning of eye care programmes. In the above context, we planned to carry out an epidemiological study to create a baseline data focused on retinopathy. We aimed to observe the prevalence of retinopathy among people with normal and abnormal glucose metabolism in a remote rural community of Northern Bangladesh and to identify the associated risk indicators for developing retinopathy in this population.
Materials and methods: This population based cross-sectional study was conducted through screening in camp settings, which included a total of 836 participants (468 male, 368 female), aged at or above 25 years. Retinopathy was determined by ophthalmoscope and fundus photography. Anthropometric measurements (BMI and WHR), OGTT, glycosylated haemoglobin (HbA_1c), blood pressure, lipid profile, serum creatinine and urine albumin-creatinine ratio (UACR) were also observed. Serum glucose (fasting and 2 hr after 75 gm glucose) was measured by glucose oxidase method, HbA_1c by high performance liquid chromatography (HPLC), total cholesterol, triglyceride and HDL were analyzed by enzymatic-colorimetric method, LDL was estimated by Friedewald's formula, serum creatinine and urine creatinine were measured by alkaline picrate method and urine albumin by pyrogallol red method. Logistic regression analysis was used with adjustment for potential confounders.
Results: The overall prevalence rate of retinopathy was 5.4% (95% CI 3.9-6.9). Moreover, the prevalence of retinopathy among the diabetic, prediabetic and nondiabetic subjects were 21.6% (95% CI 11.2-32.0), 13% (95% CI 3.4-22.6) and 3.5% (95% CI 2.2-4.8), respectively. Females (6.0%) had higher prevalence of retinopathy compared to males (4.9%). The peak prevalance of retinopathy (10.7%) was found in the older age group (above 55 years). Age, BMI, WHR, blood pressure, serum glucose (fasting and 2 hr after 75 gm glucose), HbA1c, triglyceride, total cholesterol, LDL-cholesterol, serum creatinine and UACR were significantly ($p<0.05$) higher among the subjects with retinopathy compared to those without retinopathy. The retinopathy subjects with abnormal glucose metabolism had significantly ($p<0.05$) higher BMI, blood pressure, triglyceride, total cholesterol, LDL-cholesterol, serum creatinine and UACR compared to retinopathy subjects with normal glucose metabolism. On logistic regression analysis age, BMI, abnormal glucose metabolism, hypertension and UACR were found as significant independent risk indicators for the occurrence of retinopathy in this population.
Conclusion: The data suggest that, in addition to serum glucose control in diabetic patients, screening for hypertension, general obesity, and proteinuria as well as adequate treatment of these risk indicators may prevent retinopathy in rural Bangladeshi population.
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Early-onset type 2 diabetes: high risk for premature significant diabetic retinopathy

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Background and aims: The incidence of early onset (age of diagnosis <40) type 2 diabetes (T2D) is increasing. Given the potential long duration of exposure to the deleterious diabetic milieu, this cohort is at risk of developing premature diabetes complications. Diabetic retinopathy is a significant cause of morbidity in T2D and at present, the impact of early age of diabetes onset on the burden, severity and prematurity of retinopathy complication remains unclear.
Materials and methods: A cross-sectional study using hospital diabetes register and eye screening database to identify T2D subjects and quantify se-

verity of diabetic retinopathy. Early and later onset is defined as age of T2D diagnosis below and above 40 years respectively. Severity of retinopathy is classified into background, pre-proliferative, proliferative and maculopathy. Significant retinopathy (SigR) refers to pre-proliferative, proliferative or maculopathy. Prevalence and severity of retinopathy was analysed at diabetes durations of <10, 10-20 and >20 yrs.
Results: 2516 T2D subjects were identified of whom 455 were diagnosed below age 40 yrs. Mean age of diagnosis for early and later onset cohort was 32.2 and 54.6 yrs respectively. Despite the short diabetes duration (early vs later; 6.2 vs 6.4 yrs, p=NS), early-onset T2D subjects with mean age of 38.4 yrs had substantial burden of retinopathy of all severity (26.9%), similar to later-onset cohort (29.5%) with mean age of 62.4 yrs. Prevalence of retinopathy of all severity increased with diabetes duration (p<0.05) in both cohort. Diabetes duration was a significant predictor of retinopathy complications (p<0.05). The prevalence of SigR increased substantially after 10 yrs of diabetes with both early and later onset cohort experienced similar complication burden (duration <10 yrs: early vs later; 5.7 vs 8.2%, p=NS; duration 10-20 yrs: early vs later; 26.6 vs 24.3%, p=NS; duration >20 yrs: early vs later, 45.5 vs 38.5%, p=NS) which occurred approximately 15-25 years earlier among early-onset cohort (mean age - duration <10 yrs: early vs later; 38.4 vs 62.4 yrs, p<0.05; duration 10-20 yrs: early vs later; 46.5 vs 68.6 yrs, p<0.05 and duration >20 yrs: early vs later; 58.4 vs 73.8 yrs, p<0.05). Early-onset cohort were more likely to have suboptimal glycaemic control (HbA1c >7.5%) than later-onset cohort irrespective of diabetes duration (duration <10 yrs: early vs later; 67.5 vs 58.6%, p<0.05; duration 10-20 yrs: early vs later; 82.6 vs 67.5%, p<0.005; duration >20: early vs later; 81.0 vs 71.1%, p<0.05).
Conclusion: Early onset T2D subjects have substantial risk of developing sight-threatening eye disease in later years but at an earlier age than later onset cohort. Important contributing factors include suboptimal diabetes control and prolonged exposure to diabetic milieu.

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Glycated haemoglobin as a surrogate marker for the appearance and progression of retinopathy in type 2 diabetes mellitus: systematic review and meta-analysis

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Background and aims: Glycated hemoglobin (HbA1c) is commonly employed in clinical trials as a surrogate marker of diabetes control and the risk for diabetic complications in type 2 diabetes mellitus (T2DM). To date, several trials have examined the relationship between HbA1c and microvascular complications, but no systematic review (SR) has been published. We performed a SR and meta-analysis to examine the association between HbA1c and the appearance and progression of diabetic retinopathy (DR) in T2DM.
Materials and methods: We conducted a systematic literature search in electronic medical databases (MEDLINE, CENTRAL) with highly sensitive search strategy, including over 100 terms grouped into three categories: population (e.g. "diabetes mellitus", "non insulin dependent diabetes mellitus"); surrogate (e.g. "glycosylated hemoglobin") and clinically outcomes (e.g. "retinopathy"). Observational studies (OS) and randomized, controlled trials (RCT) of retinopathy in T2DM patients that reported HbA1c level were included. Estimates were made of the adjusted relative risk (RR) of complications for an increase in HbA1c of 1%. Weighted mean differences (WMD) in HbA1c level between the case (with DR) and the control group (without DR) were also calculated. Meta-regression was used to explain heterogeneity between the studies with respect to the risk of DR. The following covariates were considered: follow-up, HbA1c level, age, disease duration, BMI, cholesterol level and blood pressure.
Results: We identified 17 trials that fulfilled the inclusion criteria, involving a total of 10 236 patients. Based on two RCT (n = 240), pooled RR for incidence of DR was calculated as 1.57 (confidence interval [CI]: 1.21-2.03; p < 0.001) for an increase in HbA1c of 1%. Meta-analysis of OS confirmed the results from RCT (RR = 1,61; CI95% [1.29-2.01]; p < 0.001). Pooled data from 5 RCT (n = 514) showed that RR of the incidence or progression of DR was 1.48 (CI95% [1.04-2.10]; p < 0.029) for an HbA1c increase of 1%. The cumulative results of five OS were similar (RR = 1,50, CI95% [1.28-1.77]; p < 0.0001. Based on data from RCTs and OS there were no significant correlation between incidence of proliferative DR and increase of HbA1c of 1%. Data from one RCT demonstrated that increase in HbA1c level of 1% increased the risk of blindness (RR = 6.20, CI95% [0.95-40.61]; p = 0.057), but this but was of borderline statistical significance. A meta-analysis of two OS (n = 238) demonstrated a lower mean HbA1c level in patients without DR compared with patients with DR (WMD = 0.43, 95%CI [0.15-0.72]; p = 0.003). Pooled results of 5 OS (n = 1874) revealed that HbA1c was significantly lower in the group without progression of DR than in the group with DR progression (WMD = 0.74, 95%CI [0.39-1.08]; p < 0.001). In meta-regression HbA1c explained the heterogeneity in 65.4%. The directional coefficient was statistically significant and equals 0.58, which means that increase HbA1c level by 1% results in increase the risk of DR by 1.79 times (CI95% [1.28-2.51]; p = 0.003). The impact of other covariates on heterogeneity between trials was small and statistically insignificant.
Conclusion: The results of our SR indicate a significant correlation between HbA1c level, and appearance and progression of DR in T2DM. Thus, HbA1c may be considered an appropriate surrogate endpoint for DR in T2DM.
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High glucose alters mitochondrial morphology and membrane potential heterogeneity in retinal pericytes

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Background and aims: Mitochondrial dysfunction is known to play a role in retinal vascular cell loss, which is a prominent lesion of diabetic retinopathy. We have previously reported that high glucose (HG) induces mitochondrial fragmentation and membrane potential heterogeneity in retinal endothelial cells, which contributes to cytochrome c release and apoptosis. Thus, we sought to determine the effects of HG on mitochondrial morphology and membrane potential heterogeneity in retinal pericytes.
Materials and methods: Bovine retinal pericytes (BRPs) were grown in normal (5mM) or HG (30mM) medium for 6 days. Both sets of cells were double-stained with MitoTracker Green FM (MTG, 125nM) and tetramethylrhodamine-ethyl-ester-perchlorate (TMRE, 8nM) and imaged using confocal microscopy. Images were analyzed for average mitochondria shape within a cell using Form Factor (FF) and Aspect Ratio (AR) values of the mitochondria. FF value of 1 corresponds to a circular, un-branched mitochondrion, and higher FF values indicate a longer, more-branched mitochondrion. AR of 1 corresponds to a circular mitochondrion, and higher AR values indicate more elliptical mitochondrion. The images were also analyzed for heterogeneity of mitochondrial membrane potential within a cell, using deviation of fluorescence intensity (FI) values for the ratio of red (TMRE) to green (MTG) dye for several mitochondria within each cell.
Results: BRPs grown in HG media exhibited significant fragmentation of mitochondria compared to BRPs grown in normal media (FF for HG: 1.85 compared to 2.51 in normal, p=0.004, AR for HG: 2.32 compared to 2.80 in normal, p=0.009). Simultaneously, the BRPs grown in HG showed greater heterogeneity of mitochondrial membrane potential compared to normal BRPs (FI deviation for HG: 224 ± 78% of normal, p<0.001).
Conclusion: Under HG condition, mitochondria of retinal pericytes display significant fragmentation and membrane potential heterogeneity. The observed mitochondrial fragmentation and increased membrane potential heterogeneity in HG could play a role in the accelerated apoptosis associated with the retinal pericytes in diabetic retinopathy.
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Enhanced thrombin formation, platelet activation in patients with diabetic retinopathy

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Background and aims: Diabetic retinopathy (DR) is the commonest microvascular complication of diabetes, and remains one of the leading causes of blindness worldwide. Postulated mechanisms of this observation include prothrombotic effects. The aim of the study was to evaluate potential prothrombotic alterations in diabetic retinopathy patients in relation to hyperglycemia, including thrombin formation, platelet activation, and fibrin network structure/function.
Materials and methods: The participants were 120 healthy subjects and 150 diabetic patients. We excluded patients with nephropathy, cardiovascular dis-

ease, and clotting disorder. On the basis of the fundus photography, the participants were divided into four groups, including normal individuals (n=120), diabetes mellitus (DM) (n=45), DM with non-proliferative DR(NPDR) (n=60), and DM with proliferative DR(PDR)(n=45). The lipid profile, C-reactive protein (CRP), glucose, insulin, platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimmer and Fibrinogen were determined using routine laboratory methods. We determined generation of thrombin-antithrombin complexes (TATs) and soluble CD40 ligand (sCD40L), a platelet activation marker, at the site of microvascular injury, together with ex vivo plasma fibrin clot permeability and lysis time.
Results: The DR patients had increased maximum rates of formation and total production of TATs (by 52.9%, P <0.01, and by 22.5%, P <0.01, respectively) as well as sCD40L release (by 19.2%, P<0.01, and by 18.3%, P<0.01, respectively) compared with those with hyperglycemia, whereas PDR patients had the highest values of TATs and sCD40L variables (P < 0.01 for all comparisons). Patients with DR had longer clot lysis time (by 21%, P<0.01) similar to that in diabetic subjects, but not lower clot permeability compared with that in normoglycemic subjects. PT and aPTT were similar for all the four groups; however, their corresponding fibrinogen levels were significantly different between PDR group and controls (4.55 ±2.12g/L vs. 3.06±1.25g/L, P<0.05). There was no difference in fibrinogen levels between NPDR group, DM group and control group.
Conclusion: Diabetic patients, with retinopathy especially with proliferative retinopathy, are associated with enhanced local thrombin generation and platelet activation, as well as unfavorably altered clot features. Our results suggested that prothrombotic alterations in diabetic patients might be implicated in the pathogenesis of diabetic retinopathy.
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Retinopathy modulates taurine transporter expression in peripheral mononuclear blood cells of type 2 diabetic patients
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Background and aims: Taurine, a semi-essential aminoacid which acts as an antioxidant, cell osmolyte, and modulator of trans-membrane calcium and glucose metabolism, is more concentrated (about 10^3-fold) in the intracellular compartment than in the extra-cellular milieu due to the action of a specific Na-dependent amino acid-transporter namely the taurine transporter (TauT), whose expression has so far been either morphologically and functionally well characterized. TauT is well represented in retinal epithelial cells where is acutely down regulated by high glucose concentrations *'in vitro'*. At the same time taurine appears to be very important in the retinal function besides its known role as modulator of glucose metabolism. However since no study has ever investigated the relationship between TauT expression and diabetes, we conceived this preliminary study to test *'in vivo'* whether TauT expression is modified in blood mononuclear peripheral cells (MPC) of patients with type 2 diabetes with or without micro/macrovascular complications.
Materials and methods: We measured plasma taurine by HPLC and TauT gene expression by real-time PCR analysis in MPC of 74 type 2 diabetic patients with or without micro/macro-vascular complications and in 44 age-and-sex matched controls. In diabetic patients, presence of retinopathy, nephropathy, neuropathy and cardiovascular disease was ascertained by appropriate clinical and instrumental investigations.
Results: Median value [interquartile range] of TauT expression, represented as arbitrary units (AU), was significantly higher in diabetic patients than in age-and-sex matched controls (2.08 [2.42]AU *vs*. 1.07 [2.68]AU; p=0.009) and was weakly related to HbA1c (r=0.29; p=0.001). As compared with uncomplicated individuals a trend toward decreased TauT expression was observed in patients with macroangioapthy (n=16; 1.16 [1.55]AU *vs* 2.24 [2.23]AU), peripheral neuropathy (n=17; 1.71[0.96]AU *vs* 2.16 [2.10]AU) or persistent micro/macroalbuminuria (n=21; 2.06 [2.35]AU *vs* 2.30 [4.20]AU; p>0.05 in all cases). Patients with retinopathy (n=23) had a significantly lower TauT expression than those who were unaffected, exhibiting a median value similar to the value of controls (1.159 [1.554]AU *vs*. 2.240 [2.226]AU; p=0.006). There was no difference in median plasma taurine levels between controls and diabetics, either with or without retinopathy (29.6 [15]µmol/l *vs.* 28.9 [17.2]µmol/l).
Conclusion: TauT gene expression in MPC is modified by type 2 diabetes, being significantly increased in patients without retinopathy, hypothesising its possible selective protective role against the development of this microvascular complication.
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Immunoexpression of the vascular endothelial growth factor and its receptors in diabetic lens
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Background and aims: The diabetic cataract is one of the causes of blindness among diabetic patients. There are many different factors leading to the cataract formation. It is known that cataract appears earlier in diabetic patients than in general population and has several morphological features. Excessive glucose oxidation, polyol pathway, hyperosmolarity, deposition of advanced glycation end products in the lens and damage of its matrix are shown to lead to lens opacity in diabetic patients. The aim of the study was to indentify the vascular endothelial growth factor (VEGF) and its receptors 1 and 2 types (VEGF-R_1 and VEGF-R_2 respectively) in lens tissue after cataract surgery carried put in diabetic patients.
Materials and methods: 10 extracted diabetic lenses were studied. The mean age of included patients was 69,9±7,69 years, all patients had type 1 and type 2 diabetes mellitus, almost all patients were treated with insulin. For the immunohistochemical staining the paraffin-embedded tissue sections (4 µm) of the formalin-fixed lenses were prepared. Before staining the slides were deparaffinized and rehydrated. The 20 min heat-induced epitope retrieval was done (for VEGF determining - with DakoCytomation Target Retrieval Solution, Ph9; for VEGF-R_1 and VEGF-R_2 - with Citrate buffer, Ph6). The sections were incubating in 10% hydrogen peroxide for 20 min to blocking the endogenous peroxidase activity. The primary anti-VEGF (monoclonal mouse anti-human VEGF, clone VG1, "DAKO") was using at a dilution range of 1:50 and applied on sections using 30 min incubation at room temperature. The primary anti-VEGF-R_1 and anti-VEGF-R_2 (rabbit polyclonal anti-VEGF Receptor 1 and rabbit polyclonal anti-VEGF receptor-2 respectively, "Novus biologicals") were using at a dilution range of 1:50 and applied on sections using 60 min incubation at 36,6°C. The colored end product were developed by using the universal secondary antibodies, detection system "EnVision", "DAKO", and following incubation with 3,3'-diaminobenzidine for 5 min in dark place.
Results: We observed immunoexpression of VEGF only in 2 of 10 diabetic cataracts on the cytoplasmic membrane of the lens fibers. There wasn't found the immunoexpression of VEGF-R_1 in lens sections. But in all lenses the VEGF-R_2 was found both on the cortical subcapsular lens fibers and lens epithelium cells. The immunoexpression was strong and higher in the superficial subcapsular lens fibers.
Conclusion: VEGF is known as the necessary factor for normal growth and development of the eye. For example, it supplies metabolic effect to the retinal pigment epithelium and also is detected in normal lens during ontogenesis. In diabetic patients the lens opacification develops earlier than among nondiabetic population. Our investigation may explain the intensive cataract formation and cortical localization of lens opacity due to the metabolic and proliferative effects of VEGF to lens epithelium via its receptor VEGF-R_2. Activation of VEGF-R_2 in lens epithelium and subcapsular lens fibers may cause excessive permeability and proliferation of lens epithelium and lead to the lens opacity.

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Characterising the development of diabetic retinopathy in the Diabetes Care System West-Friesland, the Netherlands

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Background and aims: The development and progression of diabetic retinopathy is known to be influenced by risk factors such as HbA1c, blood pressure and lifestyle variables. It is also known that the pace at which diabetic retinopathy develops, progresses or regresses is heterogeneous. Firstly, we therefore identified distinct developmental patterns of diabetic retinopathy; secondly, we assessed the patient characteristics of these patterns.
Materials and methods: A cohort of type 2 diabetes patients participating in the Diabetes Care System in West-Friesland, The Netherlands was followed for 2 to 8 years, between 1998 and 2005. The first visit was considered baseline. Annually, risk factors were measured and 2-field fundus photographs were taken with a non-mydriatic camera and graded according to EURODIAB. Latent Class Growth Analyses were used to identify distinct developmental patterns of diabetic retinopathy. Baseline characteristics of these patterns were assess with ANOVA with post hoc Bonferroni corrections and Chi-square tests or with a Kurskal Wallis test in case of skewed distribution.
Results: A total of 3392 patients were included in the study. Five clusters of patients with distinct developmental patterns of diabetic retinopathy were identified: A) patients without any signs of diabetic retinopathy, B) patients with fluctuating background diabetic retinopathy, C) patients with mild background diabetic retinopathy progressing to preproliferative diabetic retinopathy, D) patients with severe non-proliferative diabetic retinopathy progressing to (pre)proliferative diabetic retinopathy, and E) patients with persistent proliferative diabetic retinopathy. Risk factors characterizing the various patterns are shown in Table 1. Results show cluster A as the largest cluster characterized by low fasting plasma glucose levels and HbA1c and a short diabetes duration.
Conclusion: Identification of different developmental patterns of diabetic retinopathy is possible and might help understand the influence of certain risk factors on the course of diabetic retinopathy in individual diabetes patients.

Table 1. Selected baseline characteristics of five distinct developmental patterns of diabetic retinopathy (mean ± sd or median (interquartile range)).

	Cluster A (n=2955)	Cluster B (n=297)	Cluster C (n=71)	Cluster D (n=41)	Cluster E (n=28)	P < 0.05 between clusters:
Albumin-creatinine Ratio	3.2 ± 14.0	5.5 ± 17.9	6.4 ± 19.5	9.7 ± 25.1	11.2 ± 22.3	A vs. E
HDL cholesterol (mmol/l)	1.19 ± 0.32	1.20 ± 0.32	1.35 ± 0.81	1.13 ± 0.31	1.26 ± 0.35	C vs. A/B/D
Fasting plasma glucose (mmol/l)	8.8 ± 3.4	9.8 ± 3.1	9.9 ± 2.8	10.9 ± 3.6	10.7 ± 4.2	A vs. B/D/E
BMI (kg/m$^{2)}$	30.1 ± 5.3	28.8 ± 4.8	29.3 ± 4.6	30.7 ± 5.7	31.6 ± 5.6	A vs. B
Systolic blood pressure (mmHg)	142 ± 21	145 ± 22	149 ± 24	143 ± 26	149 ± 22	A vs. C
HbA1c (%)	7.6 ± 1.8	8.1 ± 1.9	8.1 ± 1.6	9.5 ± 2.1	9.0 ± 1.8	A vs. B/D/E D vs. B/C
Diabetes duration (years)	2 (1 - 5)	5 (1 - 9)	6 (3 - 12.5)	10 (4.5 -14)	13 (7.5 - 24.5)	P < 0.001

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Information technology to control screening for diabetic retinopathy

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Background and aims: Annual screening for diabetic eye disease is effective, but may be too frequent and costly for low risk patients. The aim of the study is to use computer based individual risk assessment to make diabetic eye screening programs less expensive and safer.
Materials and methods: We used epidemiological data to create a mathematical algorithm, which calculates individual risk of sight threatening retinopathy. The individual´s risk level is then used to determine his/her screening interval. The algorithm was tested against the diabetes database in Århus, Denmark (5210 patients, 20 years).
Results: In the diabetes database the algorithm (set at risk margin 4%) suggested an average screening interval of 27 months, with a range of 6 to 60 months. 95 patients progressed to sight threatening retinopathy within the recommended screening interval. At risk margin 2% the respective numbers are 17 months and 32 patients. In comparison, with the standard 12 month screening program 149 patients progressed to sight threatening retinopathy within the recommended screening interval. Our algorithm, at risk margin 4%, increases safety by 36% while reducing cost of diabetic screening programs by 55% as compared to yearly screening exams. At 2% risk margin increased safety was 79% and cost reduction 30%.
Conclusion: The use of information technology based on epidemiological data allows individual risk assessment, standardization of risk and an individualized determination of screening intervals. The reduction in screening visits decreases cost of diabetic screening programs by more than 50% compared to programs with yearly screening exams.
Supported by: Technology Development Fund

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Genomic and proteomic characterisation of non-proliferative retinopathy in a mouse model

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Background and aims: Diabetic retinopathy is the leading cause of loss of visual acuity and blindness in adulthood. Transgenic mice overexpressing Insulin-like Growth Factor (IGF-I) in the retina have retinal alterations characteristic of non-proliferative retinopathy and, with age, mice develop alterations that mimic the proliferative stage of diabetic retinopathy such as neovascularization in the vitreous cavity and retinal neovascularisation. The aim of this study was to perform genomic and proteomic analyses in IGF-I transgenic retinas to identify key molecular markers in early developmental stages of the pathology.
Materials and methods: Retinas from 4 month-old transgenic and wild-type animals were collected, homogenised and total RNA and protein extracts were obtained. We compared gene expression profiles in transgenic and wild-type retinas with microarrays and confirmed the expression of selected genes by RT-PCR. Retinal protein extracts were separated by bidimensional electrophoresis and protein spots were identified using mass-spectrometry.
Results: Gene profile analysis detected 37 genes differentially expressed, 25 of which were up-regulated and 12 genes were down-regulated more than 1.5-fold in transgenic retinas compared with wild-type. Most of the up-regulated genes were classified in three categories: gliosis, retinal stress and angiogenesis, whereas down-regulated genes were related with CNS development and angiogenesis. By RT-PCR we found that transgenic retinas already overexpressed gliosis-related genes (Gfap, S100b, Gja) at an early age (1.5 months old), when transgenic mice neither presented morphological nor biochemical alterations.This overexpression was maintained or even increased in transgenic animals with time. The same pattern was observed with retinal-stress-related genes such as Nupr1, Lcn2 and Edn2. Proteomic studies showed 37 proteins differentially produced in transgenic retinas relative to wild-type, 18 of which were increased, with 19 were decreased. The majority of the identified proteins contribute to metabolic processes.
Conclusion: Most of the alterations found in gene profile analysis in transgenic retinas have also been reported in retinas from diabetic rats and in

human diabetic retinas, suggesting that the activation of glial and stress-response genes play a key role in initiating the pathology. Overall, these results also validate the IGF-I transgenic mouse model as an excellent tool to find therapeutic targets for early stages of retinopathy and to assay new therapies.

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Metabolic fingerprints of proliferative diabetic retinopathy. An ^{1}H NMR-based metabonomic approach using vitreous humor

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Background and aims: To explore the metabolic profile of vitreous fluid from patients with proliferative diabetic retinopathy (PDR) using ^{1}H NMR based metabonomic analysis.
Material and methods: Vitreous samples from 22 type 1 diabetic patients with PDR and 22 vitreous samples from non-diabetic patients with macular hole (MH) (control group), closely matched in terms of age (46·1±9·2 vs. 45·3±11·5 years) were selected from our vitreous bank. The exclusion criteria were as follows: 1) previous vitreoretinal surgery; 2) photocoagulation in the preceding 6 months; 3) recent vitreous hemorrhage (less than 3 months before vitrectomy), macroscopic hemovitreous or intravitreous hemoglobin >5 mg/ml; 4) history of glaucoma; 5) renal failure (plasma creatinine ≥ 120 μmol/l); and 6) other chronic diseases apart from diabetes. ^{1}H NMR spectra were acquired on a 400 MHz (9·4 T) magnet interfaced to a Bruker Avance 400 spectrometer (Bruker, Rheinstetten, Germany). Data analysis included a principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). In addition, ^{1}H-^{1}H and ^{1}H-^{13}C HMQC (Heteronuclear Multiple Quantum Coherence) correlation spectra were acquired for the identification of metabolites. Furthermore, the main metabolites accounting for the differences in metabolic profile were also assessed by current biochemical methods.
Results: Lactate was the most abundant metabolite and it was higher in samples from PDR patients than non-diabetic patients (p=0.02). Glucose was significantly higher in samples from PDR patients than non-diabetic patients (p=0.03). After removing the lactate peak at 1.35 ppm, and using PLS-DA, a model was obtained which was able to correctly classify 19 out of 22 patients with PDR and 18 out of 22 controls, resulting in a sensitivity of 86% and a specificity of 81%. The main metabolites involved in this specific pattern recognition were galactitiol and ascorbic acid (AA), and they were significantly lower in PDR patients.
Conclusion: ^{1}H NMR based metabonomic analysis of vitreous fluid permits to obtain a metabolic signature of PDR. Apart from the higher abundance of lactate and glucose, significant deficits of galactitiol and AA are the main metabolic fingerprints of vitreous fluid from PDR patients.
Supported by: SAF2009-07408 (MCINN), CIBERDEM

1185

Identification of new pathogenic candidates for diabetic macular oedema using fluorescence-based difference gel electrophoresis (DIGE) analysis

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Background and aims: Diabetic macular edema (DME) is the main cause of visual impairment in diabetic patients. The aim of the present study was to explore the differential proteomic pattern of the vitreous fluid from DME patients by means of fluorescence-based difference gel electrophoresis (DIGE).
Material and methods: Samples of vitreous from 8 type 2 diabetic patients (4 with DME without proliferative diabetic retinopathy [PDR] and 4 with PDR without DME), and 8 from non-diabetic subjects with idiopathic macular hole (control group) were selected from our vitreous bank for proteomic analysis. To further confirm the potential candidates identified by DIGE eighteen additional samples (6 PDR, 6 DME and 6 MH, matched by age) were analyzed by ELISA. Exclusion criteria included photocoagulation during the preceding 6 months and recent vitreous hemorrhage or intravitreous hemoglobin higher than 5 mg/ml.
Results: Selecting an abundance ratio of 1.5-fold, p<0.05, as the threshold for the study, 4 proteins were specifically associated with DME. Hemopexin was significantly higher in the vitreous fluid of patients with DME in comparison with both control subjects and PDR patients. By contrast, clusterin, transthyretin and crystalline S were significantly decreased in the vitreous of patients with DME. The differential production of hemopexin, clusterin and transthyretin was further confirmed by ELISA. In view of the current information, hemopexin and clusterin seems to be more directly related to the development of DME. Hemopexin is the best-characterized permeability factor in steroid-sensitive nephrotic sindrome (SSNS). T-cell-associated cytokines like tumor necrosis factor-alpha (TNF-alpha) are able to enhance hemopexin production in mesangial cells in vitro and this effect is prevented by corticosteroids. It should be noted that proinflammatory cytokines have been involved in the development of DME and, therefore, hemopexin might be a mediator of the disruption of the blood-retinal barrier. Clusterin is associated with protection from apoptotic retinal cell death. Recently, it has been demonstrated that clusterin effectively inhibited vascular endothelial growth factor-induced hyperpermeability in human retinal microvascular endothelial cells (HRMECs) and in retinal vessels from streptozotocin-induced diabetic mice. Since clusterin plays an essential role in restoring tight junctions and limiting the inflammatory response after injury (two capital features in the pathogenesis of DME), it seems reasonable to propose clusterin deficit as a contributor to DME development.
Conclusion: Proteomic analysis by DIGE was useful in identifying new potential candidates involved in the pathogenesis of DME. These results could open up new strategies in the treatment of DME.
Supported by: SAF2009-07408 (MCINN) and CIBERDEM

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Retinal blood flow in patients with type 1 diabetes mellitus with and without diabetic retinopathy

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Background and aims: Proliferative diabetic retinopathy (pDRP) is a common microvascular complication in patients with long-standing type 1 diabetes (T1DM) and is oftenly associated with poor glycemic control. However, it is currently uncertain whether T1DM, and more in particular, pDRP causes hemodynamic changes in the retina. Therefore, we measured retinal hemodynamic function in T1DM patients with and without pDRP and controls.
Materials and methods: Thirty-three T1DM patients with DRP treated with panretinal photocoagulation (pDRP), 8 T1DM patients with background retinopathy (bDRP) and 32 T1DM patients without retinopathy or other microvascular complications (nDRP) were compared to 44 controls. Retinal blood flow was measured temporal and nasal of the optic disc, using Heidelberg scanning laser doppler flowmetry. Blood flow values of the right eye were used. To control for possible effects of extreme blood glucose values, the T1DM patients had to range between 4 - 15 mmol/l. Blood was drawn to determine lipid levels and HbA1c; 24-hour urine samples were collected to determine albumine:creatinine ratio. MANCOVA corrected for age and hypertension was used to determine group differences and regression analysis for determinants of changes.
Results: Overall, the T1DM group showed increased retinal blood flow as compared to controls for both the nasal and temporal locations (both $P < 0.05$). Regression analysis showed proliferative DRP, and albumin:creatinine ratio to be positively associated with retinal flow (both $P < 0.05$). In a separate analysis, the pDRP group showed significantly higher levels of flow as compared to nDRP patients and controls. Furthermore, a linear trend for retinal blood flow across groups was found (all $P < 0.05$).
Conclusion: In T1DM as compared to controls, retinal blood flow was increased, most pronounced in patients with pDRP. The significant linear trend might be an indication of increasing retinal blood flow with increasing retinopathy severity. The increased blood flow might be a compensatory mechanism for hypoxia, caused by the closure of retinal capillaries. Interestingly, the pDRP group shows higher blood flow even though this group is treated with panretinal photocoagulation. This is most likely caused by the remaining vasculopathy and reduced number of retinal vessels after photocoagulation.

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Positive effects of insulin in early pericyte loss

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Background and aims: Hyperglycaemia is a major risk factor for the typical alterations of diabetic retinopathy, such as loss of retinal pericytes and thickening of the basement membrane. Although many believe "pericyte drop-out" to be the result of glucose damage, the exact mechanism(s) underlining their degeneration has not been conclusively elucidated. Recently, a protective role of insulin from microvascular cell apoptosis was suggested. The objective of this study was to verify the effects of insulin on survival, intracellular glucose and expression of glucose transporters (GLUT 1, 2, 3, 4) in HRP cultured in intermittent high glucose (HGint).
Materials and methods: Pericytes were kept alternatively in high (28 mmol/l, HG) or normal (5.6 mmol/l, NG) glucose at 48h intervals for 8 days, with or without insulin (Ins) 100nM or 1μM. Control cells were cultured in stable NG or HG. GLUT transporter mRNA expression was determined by RT-PCR, intracellular glucose and apoptosis by ELISA, and cell proliferation by cell counts.
Results: HRP express GLUT 1, 3 e 4, but not GLUT2. GLUT1 expression was increased in intermittent HG (HGint) (+19.7% $p<0.05$ vs NG) but reduced when insulin was added to HGint (Ins100nM: -30.4%, Ins1μM: -31%, vs HGint). In contrast, GLUT4 was reduced in HGint (-48.7% $p<0.05$ vs NG) and increased in the presence of insulin 100nM (+34.6% $p<0.004$ vs HGint). GLUT3 mRNA was unchanged in all the above experimental conditions. In HGint, intracellular glucose levels were increased (+72.4% $p<0.05$ vs NG), and reduced by 1μM insulin (-68.3% $p<0.05$ vs HGint). Cell counts were reduced in HGint (-19,2% $p<0.05$ vs NG) and increased by insulin (Ins100nM: +49.5%, Ins1μM: +83.6%, $p<0.05$ vs HGint). Apoptosis increased with HGint ($p<0.001$ vs NG) and was completely normalized by insulin at both concentrations ($p<0.001$ vs HGint).
Conclusion: Insulin may influence the expression of glucose transporters in HRP and protect them from proliferation impairment and increased apoptosis induced by intermittent HG.
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Fenofibric acid activates survival signalling and prevents activation of stress kinases in human retinal pigment epithelial cells

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Background and aims: Diabetic retinopathy (DR) remains the leading cause of blindness among working-age individuals in developed countries. In the FIELD study on DR, fenofibrate reduced the frequency of first laser treatment for macular edema by 31% and for proliferative retinopathy by 30%. However, little is known regarding the molecular mechanisms by which fenofibrate exerts its beneficial effects. The aim of the present study was to explore for the first time the effects of fenofibric acid (the active metabolite of fenofibrate) on stress and survival signalling pathways in human retinal pigment epithelium (RPE) cell line under culture conditions mimicking the diabetic milieu.
Materials and methods: Human RPE cells (ARPE-19) were cultured in 5 mM or 25 mM glucose for 21 days. Then, some dishes were treated with fenofibric acid (100 μM) for a further 3 days. At day 3, cells were placed into the hypoxic chamber (1 % oxygen) for 6 or 24 h. As a control, some dishes were maintained under normoxic conditions. At the end of this experimental protocol, cells were harvested and collected for RNA or protein extraction. Immunoprecipitations, Western blot analysis and quantitative real-time PCR were performed.
Results: ARPE-19 cells cultured under hyperglycaemic conditions induced the expression of the hypoxia-inducible factor HIF-1α and the phosphorylation of stress-activated kinases (JNK and p38 MAPK). Under hypoxic conditions, the phosphorylation of JNK as well as its substrate c-Jun and p38 MAPK was increased in parallel with the induction of HIF-1α. This effect was increased by the combination of hyperglycaemic and hypoxic conditions. Cells pre-treated with fenofibric acid were protected against the activation of stress-inducible kinases by hyperglycaemia, hypoxia or both conditions. Moreover, fenofibric acid increased the survival signalling, measured by the expression and phosphorylation of insulin-like growth factor (IGF-I) receptor, IRS-1, IRS-2, Akt/PKB and p44/p42 MAPK, at 6 h of hypoxia plus hyperglycaemia.
Conclusion: The diabetic milieu triggers the activation of stress-inducible kinases in cultured ARPE-19 cells. Under this condition, fenofibric acid elicited a dual protective effect through the down-regulation of stress signalling and the induction of survival pathways. These mechanisms could be involved in the reported beneficial effects of fenofibrate on DR.
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Fenofibrate reduces fibronectin overexpression in human retinal pigment epithelial cells cultured under conditions mimicking the diabetic milieu

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Background and aims: Diabetic retinopathy is the leading cause of blindness and vision loss in the working age population. The fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study showed significant benefit of reducing the risk of microvascular complications in diabetic patients including the need for laser treatment for diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR) by 30%. However, it is unclear how fenofibrate prevents the progression of DME. Having shown that inhibition of fibronectin overexpression restores blood retinal barrier in diabetes, in this study, we examined the effect of fenofibrate on fibronectin expression in a human retinal pigment epithelial (RPE) cell line under culture conditions mimicking the diabetic milieu.
Materials and methods: ARPE-19, a spontaneously immortalized human RPE cell line, was cultured for 18 days in medium supplemented with 10% fetal bovine serum in high glucose condition (25 mM D-glucose). To study the effect of fenofibrate on fibronectin expression, 100 μM fenofibric acid was added in the last 3 days of the experiment (days 19, 20, 21) to cells grown in high glucose medium or high glucose medium plus IL1β (10 ng/ml for 2 days: days 20, 21) until the end of the experiment. The combination of high glucose + IL1β was used to provoke the disruption of the monolayer, thus mimicking the effects of the diabetic milieu. The cells were subjected to serum starvation (1% FBS) during the treatments. Fibronectin expression was evaluated by real time RT-PCR and Western blot analysis. Barrier function of RPE (permeability) was assessed by measuring apical-basolateral movements of FICT-dextran (40 kDa).
Results: Compared to cells grown in normal (5.5 mM glucose) medium, cells grown in high glucose medium or in high glucose medium plus IL1b showed significant upregulation of fibronectin mRNA expression, the latter group showing a more robust (3 fold) fibronectin upregulation. Similarly, fibronectin protein expression was also upregulated in both experimental groups compared to the control. Treatment of cells with fenofibric acid significantly reduced overexpression of fibronectin both at the mRNA and protein level in cells grown in high glucose medium or cells grown in high glucose medium plus IL1β. Tubulin and beta-actin protein levels used as controls were not altered by fenofibric acid. Treatment with fenofibric acid decreased excess permeability induced by high glucose and IL1β.
Conclusion: These results indicate that downregulation of fibronectin overexpression by fenofibric acid may have a protective effect on the leakage of the outer blood-retinal barrier. This could be one of the mechanisms involved in the beneficial effects of fenofibrate against the development of excess permeability associated with diabetic retinopathy.
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Puerarin inhibits advanced glycation end products-induced retinal pericyte apoptosis *in vitro* and *in vivo* by blocking Rac1-dependent signalling and the nuclear factor-kappaB pathway

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Background and aims: Retinal pericyte loss is one of the histopathological hallmarks of early diabetic retinopathy. Puerarin (4'-7-dihydroxy-8-beta-d-glucosylisoflavone), an isoflavone-C-glucoside isolated from *Puerarin lobata*, has various pharmacological effects, including anti-hyperglycemic and anti-inflammatory activities.
Materials and methods: In the present study, we determined the efficacy and the possible mechanism of puerarin on advanced glycation end products (AGEs)-induced apoptosis of cultured bovine retinal pericytes and retinal microvascular cells in intravitreally AGEs-modifed rat serum albumin-injected eyes of rats. We also examined the potential preventive effect of puerarin on diabetic retinopathy in streptozotocin (STZ)-induced diabetic rat. Puerarin (10 and 50 mg/kg body weight) was treated once a day orally for 16 weeks.
Results: Puerarin significantly inhibited pericyte apoptosis as well as reactive oxygen species (ROS) generation, NADPH oxidase activity and phosphorylation of Rac1 and p47phox induced by AGEs treatment. Further studies revealed that puerarin treatment remarkably suppressed the activation of nuclear factor-kappaB (NF-κB). In vivo retinal pericyte apoptosis of rats evoked by intravitreally injection of AGEs was evidently attenuated by the treatment of puerarin. In addition, the long-term administration of puerarin also prevented several histological changes, such as pericyte ghost and acellular formation of capillaries in STZ-induced diabetic rats. In fluorescein angiography, the changes of retinal vasculature (non-perfusion of fluorescein, fluorescein leakage and vessel narrowing) were significantly reduced in STZ-induced diabetic rats treated with puerarin.
Conclusion: These results demonstrate that puerarin may exert inhibitory effects on AGEs-induced pericyte apoptosis via interfering with Rac1-dependent ROS pathways and blocking NF-κB activation, thus resulting in the amelioration of diabetic retinopathy in STZ-induced diabetic rats.

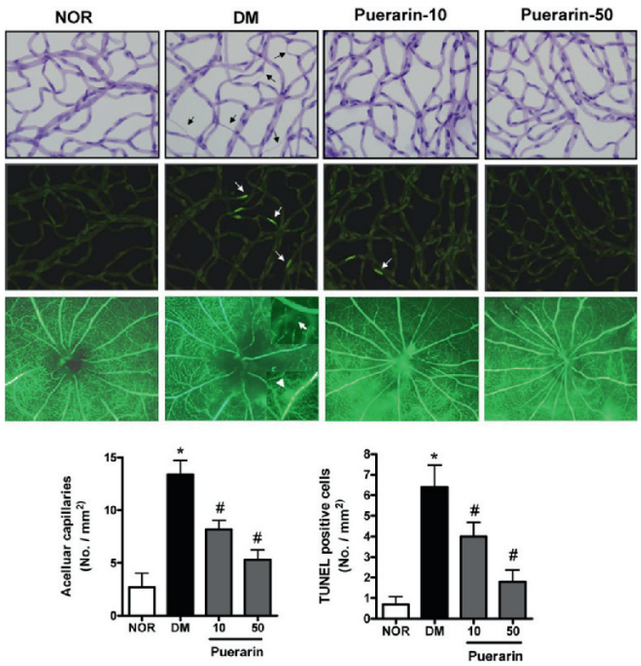

Figure 1. Effects of puerarin on diabetic retinopathy. The trypsin-digested retinal vessels from a normal rat (NOR), STZ-induced diabetic rat (DM) and diabetic rat treated with peurarin (10 and 50mg/kg, Puerarin-10 and -50) were stained with Periodic acid-Schiff and TUNEL. Acelluar capillary (black arrow) and TUNEL-positive retinal pericytes (white arrow) were observed in STZ-induced diabetic rats. In fluorescein angiography, white thick arrow and white arrowhead indicate the vessel narrowing and fluorescein leakage, respectively. All data were expressed as mean±SE. *p<0.01 vs. normal rat, #p<0.01 vs. diabetic rat.

Supported by: Korea Institute of Oriental Medicine

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Somatostatin 28 (SST-28) prevents the breakdown of human retinal pigment epithelial cells induced by the diabetic milieu

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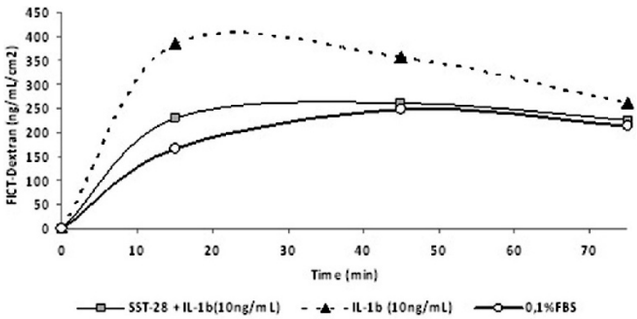
Background and aims: Diabetic macular edema (DME) results from fluid accumulation due to the breakdown of the inner and outer blood retinal barriers (BRBs). The outer BRB is formed by the tight junctions (TJs) between retinal pigment epithelial (RPE). Somatostatin (SST) has been involved in the transport of water and ions in several tissues. Various ion/water transport systems are located on the apical side of the RPE, adjacent to the subretinal space, and, indeed, a high expression of SSTR2 has been shown in this apical membrane of the RPE. In addition, we have previously demonstrated a significantly lower intravitreous concentration of SST in patients with DME in comparison with non-diabetic control subjects, being SST-28 the main molecular variant accounting for this deficit. These findings suggest that SST could have a relevant physiological role in preventing fluid accumulation within the retina, and the deficit of retinal SST observed in diabetic patients could favour the development of DME. On this basis, the aim of the study was to explore the SST effects on the outer BRB permeability in a human retinal pigment epithelial (RPE) cell line under culture conditions mimicking the diabetic milieu.
Material and methods: ARPE-19 cells (an spontaneous immortalized RPE cell line) were cultured in hyperglycemic conditions (25 mM D-glucose) for 18 days at 37ºC under 5% CO_2 in medium (DMEM/F12) supplemented with 10% fetal bovine serum. SST-14 and SST-28 ($1x10^{-7}$ M) were added to the apical side of the monolayer the last 4 days of the experiment (days 14, 15, 16 and 17) (1 application/day). Cells were also treated with IL1β (10 ng/ml) for 48 hours until the end of the experiment in order to mimic the diabetic milieu (days 16, 17). The permeability of RPE cells was determined at 18 days by measuring the apical-to-basolateral movements of fluorescein isothiocyanate (FICT) dextran (70 kDa). Lactate dehydrogenase production and cell count was used to determine putative changes in the citotoxicity or proliferation related to the different treatments.
Results: Treatment of ARPE-19 cells with SST-28 ($1X10^{-7}$) was able to prevent the increase of permeability induced by IL-1β. By contrast, treatment with SST-14 did not produce significant changes on monolayer permeability. No differences in cell number or citotoxicity were observed among the different treatments.
Conclusion: SST-28 but not SST14 has a significant protective effect on RPE disruption caused by conditions mimicking the diabetic milieu. Further investigation addressed to determining the mechanisms by which SST-28 exerts its effects in reducing permeability and their potential efficacy in DME treatment are needed.



Results of 70 kDa FICT-Dextran Permeability

Supported by: BCN Peptides S.A. and CIBERDEM (ISCIII)

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Efficacy and safety of ranibizumab monotherapy or adjunctive with laser versus laser therapy in patients with diabetic macular oedema: 12-month results of the RESTORE study

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Background and aims: Diabetic macular edema (DME) is the leading cause of blindness in diabetic patients (pts). The pathogenesis of DME is characterized by elevated levels of vascular endothelial growth factor (VEGF) in the vitreous of patients. The RESTORE study was designed to demonstrate superiority of ranibizumab 0.5mg monotherapy or as adjunctive therapy to laser photocoagulation compared to laser alone, based on mean best-corrected visual acuity (BCVA) change from baseline over 12 months in patients with DME. We present the 12-month efficacy and safety results of the RESTORE study.
Materials and methods: Randomized, double-masked, multicenter, laser controlled Phase III study of 12 months duration with ranibizumab 0.5mg in DME pts. A total of 345 pts were randomized in a 1:1:1 ratio to one of the three treatment arms: intravitreal ranibizumab 0.5mg and sham laser (ranibizumab) or ranibizumab adjunctive to laser (ranibizumab + laser) or sham injections plus laser (laser) for 12 months. The primary endpoint was the mean change of BCVA from baseline (bsl) to the average BCVA from Month 1 to 12. Treatment arm difference relative to laser were analyzed as least square means [dLSM]) using a two-sided stratified Cochran-Mantel-Haenszel test. Key secondary endpoints were mean BCVA change, safety assessed by 12-month incidence of adverse events (AEs).
Results: A total of 88% pts completed the study. The superiority of ranibizumab compared to laser was demonstrated when administered both as monotherapy and as an adjunct therapy to laser treatment with mean average BCVA changes (SD) of 6.1 (6.43), 5.9 (7.92), 0.8 (8.56), respectively (dLSM: 5.4 [ranibizumab] and 4.9 [ranibizumab + laser], both [p<0.0001]). The incidence of ocular serious AEs was low (2 pts in each of the ranibizumab + laser and laser arms). Non-ocular serious AEs were reported in 19.8% (ranibizumab), 14.4% (ranibizumab + laser) and 13.5% (laser) pts. Two deaths were reported in each treatment arm, none of which were suspected to be related to study drug or injection procedure. Ocular AEs were reported in 42.2% (ranibizumab), 43.2% (ranibizumab + laser), 38.7% (laser); eye pain being the most frequently reported ocular AE (9-10%). Non-ocular AEs were reported in 57.8% (ranibizumab), 46.6% (ranibizumab + laser) and 61.3% (laser) pts; nasopharyngitis being the most frequently reported non-ocular AE (10-14%).
Conclusion: Ranibizumab monotherapy or as adjunctive therapy with laser photocoagulation provided significantly superior benefits in BCVA as compared to laser therapy. No new safety findings were identified for ranibizumab in either arm within this study.
Supported by: Novartis

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Green tea (Camellia sinensis) ameliorates the oxidative stress and nitric oxide synthase isoforms in the retina of diabetic hypertensive rats

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Background and aims: Green tea (GT, *Camellia sinensis*), a popular beverage consumed in some parts of the world, is a rich source of polyphenols and acts as an antioxidant, antiproliferative, antitumor, and anti-angiogenic, so also may be useful to prevent diabetes in humans. A polyphenolic constituent, (-)-epigallocatechin-3-gallate (EGCG), is the major and most effective chemopreventive agent in GT. Because several lines of evidence suggest that oxidative stress and nitric oxide (NO) system contributes to the pathogenesis of diabetic retinopathy (DR), we tested the hypothesis that GT prevents retinal oxidative/nitrosative stress and thus ameliorating the early markers of DR.
Materials and methods: Diabetes was induced in spontaneously hypertensive rats (SHR) with 12 week-old. Control rats received only vehicle (citrate buffer). The diabetic SHR (DM-SHR) groups were assigned to receive or not receive, daily freshly prepared GT (13.3 g/L). After 12 weeks, the animals were euthanized and the retinas collected. The results were compared by Analysis of Variance (ANOVA) followed by Fisher's protected least significant difference test.
Results: As expected, body weight was lower and glycaemia was greater in diabetic SHR's than in non-diabetic rats ($p < 0.0001$); the systolic blood pressures were equal in all studied groups. The early molecular markers of DR were evaluated through glial reaction by expression of glial fibrillary acidic protein (GFAP) and blood retinal barrier breakdown by the estimation of the tight junction protein expression occludin. It was observed that there was a significant increase in GFAP expression (p=0.0003) and a decrease in occludin levels in retina (p=0.01) of non-treated DM-SHR group compared with control rats. Retinal oxidative damage evaluated by immunohistochemistry for 8-hydroxy-2'-deoxyguanosine (8-OHdG) and nitrotyrosine (NT) levels, were greater in diabetic than in nondiabetic rats (p<0.0001 for 8-OHdG and p=0.04 for NT). Similarly, the retinal inflammation estimated by immunolocalization of ED1/microglial positive cells was significantly higher in diabetic than in control SHR's (p=0.003). The phospho-serine isoforms of neuronal (Ser 847-nNOS) and endothelial nitric oxide synthases (NOS) (Ser 113-eNOS) were also increased in retina of diabetic SHR rats compared with control (p=0.0002 for Ser 847-nNOS and p=0.02 for Ser 113-eNOS). The Cu/Zn superoxide dismutase enzyme (Cu/Zn-SOD), an important antioxidant defense, was marked elevated only in animals which received oral treatment with GT compared with no treated groups (p=0.0006). The treatment with GT reestablished all of the above-mentioned parameters.
Conclusion: GT prevented the oxidative damage and reduced the activation of the constitutive NOS isoforms in retina from diabetic hypertensive rats. As a consequence, it was observed an ameliorating in DR indicators. These findings suggest that GT displays protective effects against retinal diabetic disease.
Supported by: State of São Paulo Research Foundation (FAPESP)

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Diabetic retinopathy before and after cataract surgery

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Background and aims: Increased retinopathy progression has been reported after cataract surgery in patients with diabetes mellitus. To assess the influence of cataract surgery on visual acuity and retinopathy progression, all diabetic patients who were subjected to cataract surgery during 2007-2009 have been followed up at the Ophthalmology Clinic.
Materials and methods: One eye of each of 70 patients was included in the study, 35 monocularly and 35 binocularly operated on. Sixteen of the 70 patients had proliferative diabetic retinopathy (PDR) at baseline. The degree of glycaemic control was assessed by measurements of HbA1c.
Results: Most patients obtained improved visual acuity; a postoperative visual acuity of 0.5 or better was achieved in 89% of diabetic surgical eyes. Progression of the retinopathy occurred in 30 out of the 70 eyes, and was associated with mean level of HbA1c (p=0.04), duration of diabetes (p=0.02), insulin treatment (p=0.001), and presence of retinopathy at baseline (p=0.01). Patients who progressed had a significantly higher incidence of macular oedema (p=0.006) than those who did not progress. No significant differences were found when operated and non-operated eyes were compared in the 35 patients with monocular surgery. Two patients in this group, however, ended up with macular oedema and worse vision in the operated eye than in the eye which was not operated on. Both patients had background retinopathy before surgery.
Conclusion: Patients in this study, also those with PDR, obtained good visual acuity, better than in most previous studies. Poor glycaemic control was a factor of importance for the progression of diabetic retinopathy after cataract surgery.

PS 118 Diabetic nephropathy: clinical observations

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Prescription of medications: Cumulative costs in outpatients with type 1 diabetes (The FinnDiane Study)

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Background and aims: Diabetes' high prevalence, chronic nature, and its association with complications increase the use and the costs of medications. The identification of subgroups of patients may give a more precise view of the real costs. The aim of this study was to estimate the cumulative costs of medications according to the complication status and duration of diabetes.
Materials and methods: The Finnish Diabetic Nephropathy Study (FinnDiane) data (N=3 721) were linked to the Drug Prescription Register (mean age 39.1±11.8 yrs, 51% men, mean duration of diabetes 30.9±11.4 yrs). 11-year cumulative costs of medication for each patient were calculated between 1998 and 2008. Costs were inflated to year 2008 Euros using the Consumer Price Index. Patients were divided in 10-year groups according to the duration of diabetes in 1998. Data on macrovascular diseases (MVD) and progression to end stage renal disease (ESRD) were retrieved from follow-up visits, medical files, or death certificates for all patients until 2008. One quarter of patients (n=883) had MVD (stroke, AMI, CHD, coronary revascularization, amputation) and/or ESRD (dialysis, kidney transplantation). Based on the complication status the patients were divided into 4 groups: no MVD or ESRD, MVD only, ESRD only, and both MVD and ESRD. Generalized linear mixed models were used to evaluate the 11-year cumulative costs. Costs were adjusted for age, sex, duration of diabetes, total insulin dose/day and body mass index, complication status and contributing years.
Results: The observed cumulative medication costs were 11 000 (no MVD or ESRD), 15 200 (MVD only), 80 900 (ESRD only), and 67 200 € (both MVD and ESRD) in the respective complication status groups. The average costs per year were 1 000, 1 600, 8 000, and 7 500 €, respectively. On average men's costs were 16% higher than women's. After adjustment, the cumulative costs of medications were 65% higher when MVD was present (increased from 11 600 to 19 000 € per patient). Notably, costs increased substantially when ESRD was present, being 7.5 times higher and, when antidiabetic medications (ATC A10) were excluded, even 22 times higher. The costs were approximately 10% lower when both MVD and ESRD were present probably due to the high mortality rate in this group (54% of the patient died during 1998 - 2008) and hospitalisation (inpatient medications costs were not included). The costs of antidiabetic medication remained rather stable, irrespective of complication status or duration of diabetes. However, when complications were present these costs were markedly lower in all 10-year duration groups. Without complications the costs of medications related to comorbidity (other than ATC A10) were rather low in all duration groups (3 000 - 5 700 €). In contrast, with complications these costs increased remarkably.
Conclusion: The cumulative costs of medications increased substantially when ESRD was present. Since no considerable differences were observed in the costs of antidiabetic medications, the observed increase was entirely due to the increase in the costs of medications related to comorbidity.

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Cardiovascular risk factors differ between type 2 diabetic patients with and without renal impairment

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Background and aims: Diabetes, albuminuria and renal impairment are all major determinants of cardiovascular disease. The aim of this cross-sectional study was to assess potential differences in cardiovascular risk factors in type 2 diabetic patients with and without renal impairment. This was done in National Diabetes Register (NDR), a large nation-wide population-based diabetes register.
Materials and methods: 62 661 patients with T2D aged 18-80 years with complete datasets on albumin excretion, renal function (serum creatinine) and clinical characteristics reported to the Swedish National Diabetes Register in 2008 were included. Albuminuria was defined as urinary albumin excretion rate > 20 μg/min and renal impairment as estimated glomerular filtration rate; eGFR< 60 ml/min/1.73 m2 according to MDRD. Values are given as crude means and standard deviations (SDs). In addition, data was analysed with least square (LS) means and frequencies, standard errors (SE) for clinical characteristics, comparing patients with renal impairment and those with no renal impairment at GLM regression adjusting for all other variables. P-values for each variable are given after these adjustments.
Results: 15% of all patients had renal impairment (n=9 308) and 58% of these patients were non-albuminuric. Several differences in cardiovascular risk factors were found between patients with and without renal impairment. Patients with renal impairment were older (71.2±6.7 vs. 64.0 ±9.3), had a longer diabetes duration (11.1±7.7 vs. 7.8±6.4 years), were more often women (50 vs. 40%), had significantly lower total- and HDL-cholesterol (4.6±1.0 vs. 4.7±1.0 and 1.23±0.4 vs. 1.28±0.4 mmol/L, respectively), higher triglycerides (2.0±1.2 vs. 1.8±1.1 mmol/L), higher HbA1c (7.1±1.1 vs. 7.0± 1.1 % (DCCT)), higher BMI (30.2±5.3 vs. 29.7±5.2 kg/m^2) and higher systolic blood pressure (138±18 vs. 137±16 mmHg). In addition, fewer patients with renal impairment performed physical activity >3 times a week (44 vs. 52%) and a smaller proportion were smoking (10 vs. 15%) (All p-values <0.001) compared to patients without renal impairment. When patients with renal impairment were compared with those without renal impairment at GLM regression adjusting for all other variables similar relationships were found for all variables except for HbA1c and systolic blood pressure where adjusted values were lower in patients with renal impairment (7.0±0.01 vs. 7.1± 0.01 % (DCCT) and 135±0.2 vs. 137±0.1 mmHg) (adjusted LS means±SE).
Conclusion: The majority of patients with type 2 diabetes and renal impairment were non-albuminuric. Several differences in cardiovascular risk factor pattern were found between type 2 diabetic patients with and without renal impairment. Interestingly, patients with renal impairment had better glycemic control and blood pressure when adjusting for all other variables. This finding should be further investigated. The cause-effect relationship and potential treatment effects could not be assessed in this cross-sectional study and thus prospective studies are warranted.

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Relation between echocardiography and coronary artery disease in asymptomatic type 2 diabetic patients with elevated urinary albumin excretion rate

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Background and aims: Coronary artery disease (CAD) is the major cause of morbidity and mortality in type 2 diabetic patients, especially in patients with elevated urinary albumin excretion rate. Left ventricular (LV) hypertrophy and systolic/diastolic abnormalities has been suggested as part of the diabetic cardiomyopathy, but relation to CAD is unclear. This study examined echocardiographic parameters, including LV mass index, LV systolic and diastolic function, and their relation to screen detected previously undiagnosed CAD in type 2 diabetic with urinary albumin excretion rate (UAER) >30mg/24h.
Materials and methods: The study included 200 type 2 diabetic patients without prior clinical CAD. Patients with plasma NT-proBNP >45.2 ng/L and/or coronary calcium score >400 were arbitrarily stratified as high risk patients for CAD (n=133), and all other patients as low risk patients (n=67). High risk patients were examined by myocardial perfusion imaging (MPI; n=109), and/or CT-angiography (CTA; n=20), and/or coronary angiography (CAG; n=86). LV systolic and/or diastolic dysfunctions were evaluated in all patients by conventional echocardiography and tissue Doppler imaging. Moderate-severe LV hypertrophy was defined by LV mass index >131 g/m^2 in men and >108 g/m^2 in women.
Results: Patients received multifactorial treatment, yielding mean (SD) HbA_{1c} 7.9 (1.3)%, plasma total cholesterol 3.9 (0.9) mmol/L and arterial blood pressure 130 (17)/75 (11) mmHg. The LV mass index was 87 (21) g/m^2 and 8 (4%) patients had moderate-severe LV hypertrophy. LV systolic function was well preserved (LV ejection fraction 59 (5)%) and impaired (<50%) in only 5% of patients. LV diastolic dysfunction (LVDD) was found in 109 patients (54.5%),

of whom 7 (3.5%) had impaired relaxation and 102 (51%) had a pseudonormal pattern of LV filling. In 70 high risk patients, significant CAD was demonstrated by MPI, and/or CAG. In a multiple regression model, the adjusted odd ratio (OR, 95% CI) for having significant CAD was 2.91 (1.41-6.00) in patients with LV mass index above the median. Patients with right atrial volume above the median (>49.1 ml) had higher risk of CAD (unadjusted OR 2.20 (1.06-4.56) than other patients. LVDD was not associated with LV mass index or significant CAD but was associated with poor metabolic control (adjusted OR 1.46 (1.14-1.87) per 1% increase of HbA_{1c} (p=0.003)).
Conclusion: In our study of asymptomatic type 2 diabetic patients with elevated UAER but receiving multifactorial treatment, the prevalence of abnormal LV mass and function was low. However, even within normal range LV mass index was an independent predictor of significant CAD. LVDD was common but not associated with CAD.
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Changes in skin microcirculation and large arterial vessels in patients with diabetic nephropathy
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Introduction: Diabetic vascular complications are divided into microangiopathies (pertaining to microvasculature) and macroangiopathies (pertaining to large vessels) that lead to the development of atherosclerosis that clinically manifests as ischemic heart disease or cerebral stroke. Increasing vessels stiffness and intima-media thickness (IMT) are indicators of macroangiopathy progression. Abnormalities of microcirculation are the basis of microvascular complications in diabetes and present in many organs. The doppler laser method allows for direct evaluation of skin microcirculation.
Aim: Study aim was to assess changes in the macro - and microcirculation in patients with diabetic nephropathy, as well as the influence of nephroprotective therapy on the evaluated parameters.
Materials and methods: 70 patients diagnosed with diabetes mellitus type II were studied: group 1- 48 patients with nephropathy, group 2 - 22 patients with diabetes mellitus without vascular complications (comprising the control group), and group 3- 25 patients with nephropathy, examined 36 months post intensive nephroprotective therapy. Visual diagnostic procedures evaluating macro- and microvessels were performed in all patient groups using usg (IMT) and laser doppler [skin mean basal flow (MFb), post occlusion flow (PF)].
Results: In the group with diabetic nephropathy, significantly higher values of PWV, as well as IMT were noted in cervical arteries when compared to the control group (p<0.01). In this group, significantly slower flow in microcirculation (MFb) at rest was noted (p< 0.01); as well as post occlusion (PF) (p=0.05) when compared to the group without complications. After 36 months of nephroprotective therapy in the studied group - stabilization of renal function was found and no substantial differences were noted in aortic pulse wave velocity (AoPWV) and also in IMT when compared to initial values at the beginning of the study. However, improvement was noted in skin microcirculation parameters when compared to initial values (p= 0.05); as well as a trend for improvement of maximal flow post occlusion (p= 0.09) and at the temperature of 44°c (MF44) (p= 0.05). Results are presented in the table.
Conclusion: Study results point to an existence of advanced atherosclerotic changes and decreased microcirculation skin flow in the group of patients with nephropathy than in the patient group without diagnosed nephropathy. After 36 months of observation and intensive nephroprotective therapy - stabilization of macrocirculation changes and regression of skin microcirculatory abnormalities were observed.

*p<0,01

Parameter	Study group	Control group	Study group after 36 month
AoPWV m/s	14.1±2.9*	11.8±2.1*	13.5±2.1
IMT-L mm	0.87±0.22	0.81±0.22	0.9±0.2
IMT-P mm	0.86±0.2*	0.77±0.21*	0.9±0.2
MFb PU	7.7±5.1*	12.1±7.1*	10.5±8.1*
PF PU	27.4±22.2*	30.8±10.7*	33.7±24.9*
MF44 PU	88.5±46.8*	94.6±47.3	103.9±67.1*

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Serum uric acid is related to cardiovascular events and correlates to NT-proBNP and albuminuria in patients with diabetes mellitus
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Background and aims: Hyperuricemia is a risk factor for cardiovascular events and renal insufficiency. It correlates to intima media thickness and microalbuminuria. The aim of this study was to evaluate the relationship between NT-proBNP as an established marker for subclinical cardiovascular disease and uric acid in patients with diabetes mellitus.
Materials and methods: In a prospective observational study we recruited 494 patients with diabetes mellitus. Serum uric acid, NT-proBNP, urinary albumin to creatinine ratio and HbA1c as well as other cardiovascular risk factors were evaluated at baseline. Patients were then followed for 12 months and hospitalisations due to cardiac events (ischemic heart disease, rhythm disturbances, heart failure) were recorded.
Results: The mean duration of diabetes was 13 ± 11 years. Patients were 60 ± 13 years old and mean HbA1c was 7.7 ± 3.2%. At baseline mean uric acid was 5.3 ± 1.6 mg/dl, NT-proBNP was 248 ± 412 pg/ml and mean urinary albumine to creatinine ratio was 96 ± 361 mg/g; Uric acid significantly correlated to NT-proBNP (r= 0.258 p < 0.001) and urinary albumin to creatinine ratio (r = 0.198 p< 0.001). In a logistic regression model including the variables uric acid, NT-proBNP, systolic blood pressure and urinary albumin to creatinine ratio, NT-proBNP was the best predictor of cardiac events (Hazard Ratio 1.002, Wald 37.2 p < 0.001). In a second step uric acid provided additional prognostic information (Hazard Ratio 1.353 Wald 7.0 p < 0.05).
Conclusion: Serum uric acid is a predictor of cardiac events and correlates to NT-proBNP underscoring the importance of uric acid as a cardiovascular risk marker in patients with diabetes mellitus.

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Serum osteoprotegerin is related coronary artery calcification, urinary albumin excretion, and diabetic retinopathy in Japanese patients with type 2 diabetes
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Background and aims: Osteoprotegerin (OPG), a secreted glycoprotein identified as an inhibitor of bone resorption, has recently been indicated to act as an important regulatory molecule in the vasculature. Recent studies also suggest that serum OPG levels are associated with endothelial dysfunction, coronary artery calcification (CAC), and micro- and macroangiopathy in Type 2 diabetes, and, in addition, diabetic microangiopathy is correlated with macroangiopathy. However, a mechanism(s) underlining a relationship between diabetic macro- and microangiopathy is unclear. Therefore, we have measured serum OPG levels and cross-sectionally investigated relationships among OPG levels, macroangiopathy, and diabetic microangiopathy (retinopathy and nephropathy) in 82 Japanese patients with type 2 diabetes mellitus.
Materials and methods: Blood samples were obtained in fasting state. Variables analyzed were age, sex, blood pressure, BMI, waist/hip ratio, daily blood glucose profile, M value and MAGE as markers of blood glucose fluctuation, HbA1c, glycated albumin (GA), serum levels of OPG, IRI, TC, TG, HDL-C, LDL-C, Lp(a), uric acid (UA), homocysteine, adiponectin, leptin, and PAI-1, and urine C-peptide (CPR), surrogate markers of macroangiopathy [carotid IMT and plaque, pulse wave velocity (PWV), ankle-brachial index (ABI), and CAC score (CACS)], and presence of microangiopathy [DR > simple retinopathy, and urine albumin excretion (UAE) as a marker of nephropathy].
Results: Serum OPG levels were positively correlated with age, DM duration, systolic BP, PWV, log(CACS+1), BUN, logUAE, and presence of DR, whereas inversely with U-CPR, and postprandial glucose excursion. Multiple regression analysis showed that significantly independent predictors for the OPG levels were systolic BP and DR. Log(CACS+1) tended to be significant. On the next step, we analyzed focusing on an association of macroangiopathy [log(CACS+1)], and micropangiopathy (DR and logUAE) with the serum levels of OPG. Log(CACS+1) was positively correlated with OPG levels, age,

waist, systolic BP, PWV, IMT, BUN, UA, postprandial glucose excursion, and MAGE, whereas inversely with ABI. Multiple regression analysis showed that independent predictors for log(CACS+1) were OPG, DR, age, and waist. In comparison with patients without DR, those with DR had significantly higher levels of serum OPG, DM duration, PWV, UA, and log(CACS+1), whereas significantly lower levels of HDL, U-CPR, and diastolic BP. Logistic analysis revealed that independent predictors for DR were OPG levels, diastolic BP, GA, and log(CACS+1). Finally, logUAE was positively correlated with OPG, SBP, PWV, UA, LDH, and postprandial glucose excursion. Multiple regression analysis showed that independent predictors for logUAE were OPG, ABI, PWV, and MAGE.
Conclusion: Thus, these results indicate serum OPG level was an independent predictor for CAC, DR, and UAE, and vice versa in Japanese patients with type 2 diabetes. The results imply that there may be a bilateral interaction between coronary artery atherosclerosis, diabetic retinopathy and nephropathy in Type 2 diabetic patients.

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Chronic cigarette smoking could contribute to diabetic nodular glomerulosclerosis
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Background and aims: Nodular glomeruloclerosis, glomerulomegaly, thickening of glomerular basement membrane and arteriolar hyalinosis are present both in diabetic nephropathy with nodular glomerulosclerosis and in idiopathic nodular glomerulosclerosis, which suggests a common mechanism. Chronic smoking is known as a risk factor in the former and a potential causative factor in the latter disease. We hypothesized that there are more smokers among patients with diabetic nephropathy with glomerulosclerosis (DNP + NGS), than among patients with diabetic nephropathy without nodular glomerulosclerosis (DNP without NGS).
Materials and methods: A retrospective analysis of all native renal biopsy specimens (n=890) available in the Renal Pathology Laboratory at our clinic from 2002 to 2009 was performed. The characteristics of patients were collected from medical documents and the smoking habits were confirmed by a questionnaire.
Results: The data revealed significantly more smokers (10 out of 11) among patients with diabetic nephropathy and nodular glomerulosclerosis compared to the random selected patients (4 out of 10) with diabetic nephropathy without histological signs of nodular glomerulosclerosis (p=0.024). Between the two group of patients (DNP + NGS and DNP without NGS) no significant difference was found in the age (56±14.7 vs. 53.7±7.7years, p=0.875), body mass index (30.5±5.6 vs. 31.9±4.1 kg/m2, p=0.578), duration of diabetes mellitus (12.11±5.67 vs. 8.89±6.51 years, p=0.279), prevalence (89% both) and duration (10.43±8.92 vs. 12.25±13.55 years, p= 0.573) of hypertension, serum total cholesterol (7.5±5.3 vs. 6.9±2.2mmol/l, p=0.781), serum triglyceride (4.5±4.4 vs. 2.8±1 mmol/l, p=0.344), serum creatinine (225.6±136.2 vs. 180.6±88.1, p=0.479), estimated (MDRD) renal function (39.51±24.58 vs. 45.24±26.92ml/min, p= 0.685) and the renin-angiotensin system blocker treatment (100% vs. 75%, p=0.155) at the time of the kidney biopsy.
Conclusion: Our results show, that chronic cigarette smoking could be a potential cause of nodular glomerulosclerosis seen in diabetic nephropathy patients.

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Erythropoetin therapy affects HbA_{1c} levels in patients with diabetes mellitus and chronic kidney disease not on haemodialysis
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Background and aims: Glycated haemoglobin (HbA1c) is the most widely accepted and used method of assessing chronic glycaemia in patients with diabetes mellitus (DM). Treatment of anaemia in patients with chronic kidney disease (CKD) using erythropoietin stimulating agents (ESAs) has resulted in significant improvements to quality of life and levels of anaemia without the need for blood transfusions . Although some studave shown a fall in HbA1c in patients treated with ESA therapy it is not known whether there is a change in mean blood glucose. This study has therefore sought to establish the effect of ESA therapy on both HbA1c and mean blood glucose amongst a group of patients with diabetes and known CKD.
Materials and methods: This was a prospective study of patients with DM and CKD stage III or IV selected for treatment with erythropoietin stimulating agents (ESA) from Jan 2009 to December 2009 inclusive. All patients were requested to perform 7 point glucose monitoring (7PGM) 3 times weekly for a month before commencement of ESA until the end of the study. Continuous glucose monitoring (CGMS) was performed measurements of interstitial glucose levels were made. Mean blood glucose (MBG) of each patient was calculated by averaging daily capillary glucose readings on days where patients had more than 3 readings a day or more and discerning the results of the CGMS.
Results: There were 15 patients (11M 4F, median age 70 (IQR 62,75)) with Type 2 DM. The mean follow up time of was (mean±SD) 17.3±3.3 weeks. There was a statistically significant rise in haemoglobin and haemotocrit levels following ESA therapy and no significant change in the eGFR. The HbA1c levels fell without discernible alteration in glycaemic control. The mean HbA1c fell from 7.3 to 6.6% (p=0.001, paired t test). This change occurred in the presence of a MBG which remained unchanged (8.7 mmol/L vs 8.7 mmol/L, p= 0.89).
Conclusion: Anaemia is a common phenomenon in patients with CKD. This study confirms the fall in HbA1c as a result of ESA treatment and proves that this is independent of a change in glycaemia. In these patients, therefore, alternative markers of glycaemia are needed to accurately assess their glucose control.

Patients on ESA therapy

	Before ESA mean (95% CI)	After ESA mean (95% CI)	p (paired t test)
HbA1c(%)	7.31 (6.42,8.54)	6.63 (6.03,7.36)	0.01
Hb (g/dl)	9.52 (9.18,9.86)	11.51 (11.15,11.85)	<0.01
Haematocrit	0.324 (0.296,0.350)	0.378 (0.341,0.398)	<0.01
Mean blood glucose (mmol/L)	8.72 (7.31,10.12)	8.78 (7.47,9.99)	0.89

1203

Irbesartan treatment does not influence plasma levels of the advanced glycation end products CML and CEL in patients with type 2 diabetes and microalbuminuria. An IRMA2 substudy
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Background and aims: Guidelines state that patients with type 2 diabetes who develop microalbuminuria should be treated with angiotensin receptor blockers (ARBs). In vitro studies and animal experiments have shown inhibiting effects of ARBs on advanced glycation end products (AGEs), which are known to be involved in the development of cardiovascular complications in diabetes. However, human data to confirm such beneficial effects of ARBs are lacking.
Materials and methods: We analysed data from a multicentre, double-blind, randomised controlled trial in patients with type 2 diabetes and microalbuminuria, the primary goal of which was to examine the renoprotective effects of irbesartan (150 or 300 mg once daily). Secondary endpoints included, among others, measures of the plasma levels of the AGEs N^{ε}(carboxymethyl)lysine (CML) and N^{ε}(carboxyethyl)lysine (CEL) in the treatment arm receiving 300 mg irbesartan (n =139) and in the placebo group (n =125). Effects of treatment at 1- and 2-year follow-up were analysed by means of generalized estimating equations.
Results: Levels of CML and CEL (as well as all other patients' characteristics) did not differ between groups at baseline. No significant changes were observed in CML and CEL over time in either group and there was no effect of treatment at any time-point. Mean differences between groups over time were -0.96 nM/mM lysine (95%CI: -3.43; 1.51) for CML and -0.10 nM/mM lysine (95%CI: -0.76; 0.56) for CEL (Figure 1).
Conclusion: Long-term irbesartan treatment does not influence plasma levels of the AGEs CML and CEL in patients with type 2 diabetes and micro-

albuminuria. These findings do not support the concept that ARBs inhibit the process of advanced glycation in humans.

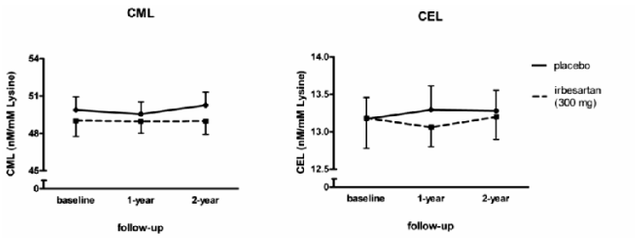

Figure 1. Means and standard errors of plasma levels of CML and CEL at baseline, 1-year and 2-year follow-up for the irbesartan treatment group and the placebo group

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PS 119 Nephropathy - role of renal function

1204

Direct correlation between initial glomerular filtration rate and long-term urinary albumin excretion in patients with type 1 diabetes

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Background: Increased glomerular filtration pressure and glomerular filtration rate (GFR) occur early in type 1 diabetes although their significance in the pathogenesis of diabetic nephropathy remains controversial.
Aims: To examine the relationship between initial GFR and urinary albumin excretion after at least 10 years follow-up.
Methods: 77 male patients (aged 18-42) with type 1 diabetes of short duration (4-8 years) and normal urinary albumin excretion (albumin: creatinine ratio (ACR)< 2.5 mg/mmol on 3 occasions) were studied at baseline using inulin clearance (ICL) to assess GFR. At baseline, 13 patients had evidence of glomerular hyperfiltration (ICL>145 ml/min/1.73m^2). Mean HbA1c: 8.3%. All patients were invited to attend a follow-up study after a median of 163 (151-168) months where they provided 3 consecutive early morning urine samples for ACR.
Results: Complete data collection was available for 12 patients; the others were either untraceable or declined to participate in the follow-up study. At follow-up, all were normotensive (mean BP: 127/75), with normal renal function; mean HbA1c 7.6%; eight patients were taking statins and 3 were on ACE-inhibitor drugs. One patient had developed overt microalbuminuria. A significant correlation was shown between baseline ICL and follow-up mean (log) ACR (r^2=0.4; p=0.028). Baseline and follow-up HbA1c were closely correlated (r^2=0.52; p=0.008). When HbA1c was entered as a covariate, the correlation between ICL and ACR just failed to achieve significance (p=0.055).
Conclusion: The results of this study support a link between GFR measured by inulin clearance and the development of nephropathy reflected by urinary albumin excretion. The relationship is, however, strongly influenced by the prevailing glycaemic control.
Supported by: UK CRN

1205

High-normal albuminuria and cardiovascular risk factors in patients with type 2 diabetes and no evidence of kidney impairment

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Background and aims: Albuminuria in the 'high-normal' range, is a predictor of cardiovascular morbidity and mortality. Which factors account for this increased risk and whether such a prediction is maintained also in the absence of a concomitant reduction of glomerular filtration rate (GFR) is unclear yet. The aim of the present study was to explore, in a large cohort of patients with type 2 diabetes (T2DM) with normoalbuminuria and no evidence of kidney impairment, the association between traditional cardiovascular risk factors and urinary albumin excretion.
Materials and methods: This was a cross-sectional study investigating 1148 (556M/592F) patients with T2DM, age 60.4 ± 10.1 yrs, duration of diabetes 10.5 ± 9.1 yrs, with normoalbuminuria [ACR 0.80 (0.01 - 3.49) mg/mmol] and estimated-GFR ≥ 60 ml/min/1.73m^2 (89.5 ± 20.8 ml/min/1.73m^2). Normoalbuminuria was defined if the albumin/creatinine ratio (ACR) was <2.5 in men and < 3.5 mg/mmol in women. Estimated-GFR was derived by serum creatinine.
Results: ACR significantly and independently correlated with gender (p=0.016), age (b=0.112, p=0.001), HbA1c (b=0.137, p <0.0001), BMI (b=0.106, p=0.036) and SBP (b=0.076, p=0.013). A gradual increase by tertiles of ACR in the proportion of patients with HbA1c ≥ 7% [269 (70.4%) vs 305 (79.6%) vs 313 (81.7%), p<0.0001], hypertension [282 (73.8%) vs 286 (74.6%) vs 322 (84.0%), p =0.020], Metabolic Syndrome [291 (76.1%) vs 298 (77.8%) vs 324 (84.5%), p=0.021] and retinopathy [83 (21.7%) vs 120 (31.3%) vs 128 (33.4%), p=0.001] was also observed.

Conclusion: Our data indicate that patients with T2DM and albuminuria in the high-normal range, even in the absence of GFR reduction, are characterized by the presence of several cardiovascular risk factors and suggest they may deserve a careful clinical follow-up. Prospective studies are needed to investigate whether a new threshold to define microalbuminuria and to be used as cut-off in the stratification of global cardiovascular risk is needed.

1206

Association of renal function with anaemia in diabetic kidney disease

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Background and aims: Anemia is a common complication of chronic kidney disease (CKD) particularly in patients (pts) with diabetic kidney disease (DKD). In DKD anemia occurs early and is more severe than in non diabetic CKD. The aim of the study was to estimate the prevalence of anemia in diabetes mellitus (DM) pts with and without renal damage.
Materials and methods: A total 2015 DM type 1 (DM 1) (n=807; 40%) and type 2 (DM 2) (n=1208; 60%) pts were screened for presence of anemia. Their mean clinical data: age - 50,0±16,1 years, DM duration - 12,2±8,6 years, $HbA1_c$ - 8,7±2,0 %, hemoglobin (Hb) - 134,8±17,8 g/l. Mean glomerular filtration rate (GFR) was calculated using the MDRD formula - 107,6±39,8 ml/min/1,73 m^2, The 66.3% pts had arterial hypertension. Anemia was defined as Hb < 13.0 g/dl for men and Hb < 12.0 g/dl for women by the gender specific definition of WHO for pts without DKD and Hb < 13.5 g/dl for men and Hb <12.0 g/dl for women by the definition of anemia in CKD by National Kidney Foundation/Kidney Disease Outcome Quality Initiative (NKF/KDOQI) for pts with DKD. Evaluation of the distribution of anemia was based on 5 stages of CKD categories according NKF/KDOQI. Patients with GFR<15 ml/min/1,73 m^2 (5 stage of CKD) and treated by erythropoesis-stimulating agents were not included.
Results: The prevalence of anemia in DKD pts (n=971; 48.2%) was 32.4% compared to 13.8% in pts without renal damage (n=1044; 51.8%) (p<0.001). In DM 1 with DKD, the anemia prevalence was significantly higher than in DM 2 (41.9% and 23.7%, respectively (p<0.001)). Comparison of anemia prevalence based on pts gender did not find significant discrepancy in total group of DKD pts and pts DM 1 with DKD, except in pts DM 2 (male - 30.5% vs female - 21.9%, respictevely (p<0.05). The prevalence of anemia significantly increased in pts with evident renal injury and achieved to 47.1% in proteinuric pts (n=323), that greatly higher compared to pts with microalbuminuria - 25.7% (n=607) (p<0.001) and normoalbuminuria - 14.3% (n=1085) (p<0.001). Anemia prevalence significantly increased when the renal failure progresses (Table 1; *P<0.05; **P<0.01 between CKD 1 stage and other CKD stages). In DKD the Hb had strong association with GFR (R=0,41; p<0,001), DM duration (R=-0.27; p<0.001). Independent factors for Hb level by multiple logistic regression analysis were DM type - 1 (beta = -0.21), gender - male (beta = -0.30), albuminuria (beta = -0.10), hypertension (beta = -0.08) and GFR (beta = 0.33) (p<0.001).
Conclusion: Anemia is a prevalent finding in pts with DKD, especially in DM 1. In half of DM 1 pts and in one-of third type DM 2 pts anemia develops when the mild decrease of GFR (60-89 ml/min/1,73 m^2). The prevalence of anemia in DKD is clearly related to the degree of albuminuria and decreasing of renal function.

Anemia prevalence in diabetic kidney disease patients according to CKD stages (%)

	CKD 1 stage (GFR ≥ 90 ml/mi/1,73 m^2), n=523	CKD 2 stage (GFR 60-89 ml/min/1,73 m^2), n=231	CKD 3 stage (GFR 30-59 ml/min/1,73 m^2), n=169	CKD 4 stage (GFR 15-30 ml/min/1,73 m^2), n=48
All patients (n=971)	21.1	35.9**	46.7**	87.5**
Type 1 (n=403)	25.5	46.5**	66.1**	85.3**
Type 2 (n=568)	18.2	27*	36.4**	92.8**

1207

Renal perfusion is reduced in apparently uncomplicated type 1 diabetic patients

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Background and aims: Magnetic resonance imaging (MRI) offers novel, non-invasive techniques for investigation of diabetic nephropathy. We assessed renal perfusion in control subjects and Type 1 diabetic patients with and without microalbuminuria.
Materials and methods: 8 Type 1 diabetic patients (T1C) (age 40.1±6.7 years, duration diabetes 28.4±6.2 years, eGFR 76.1±8.24 ml/min/1.73 m^2, albumin: creatinine ratio (ACR) persistently <2.5 mg/mmol, BP <130/80 mmHg on no treatment, no or minimal background retinopathy), 8 Type 1 diabetic patients with microalbuminuria (T1M) (ACR persistently >5.0 mg/mmol), eGFR 78.1±9.9 ml/min/1.73 m^2 ,age 41.2±7.3 years, duration diabetes 27.3±5.6 years, all with significnat retinopathy, and seven healthy control subjects (C), age 39.0±7.9 years, on no medication, participated. Six T1M participants were studied on (T1Mon) and after 4 weeks off (T1Moff) RAAS inhibition. Blood pressure (BP) control off RAAS inhibition was maintained by non-RAAS agents. Studies were performed fasting. Blood glucose was maintained at 4.0-6.0 mmol/l in the diabetic subjects by IV insulin infusion. Phase-contrast angiography was performed to measure renal artery flux over the cardiac cycle before and after water loading (20 ml/kg body weight), when urine flow >8 ml/min.
Results: Renal artery flow rate (RAF), corrected for body surface area and pulse rate, was higher in the control subjects (791±253 ml/min/1.73 m^2 body surface area) compared to T1C (622±128) and T1Moff (580±188; ANOVA p=0.05), with no significant difference between T1C and T1Moff. There was no change with water loading. RAF (587±118 vs 580±188 ml/min/1.73m^2) and eGFR (71.1±10.4 vs 78.1±9.9 ml/min/1.73m^2) were similar in T1Mon and T1Moff. RAF correlated with eGFR in all diabetic patients (r=0.59, p=0.031), and in T1C (r=0.84, p=0.01) but not in T1Mon (r=0.39, p=0.52) or T1Moff (r=0.008, p=0.99).
Conclusion: In longstanding apparently otherwise healthy Type 1 diabetic patients with clinically normal renal function, renal perfusion is decreased to a level similar to that in Type 1 microalbuminuric patients. Perfusion is unaltered by water diuresis or RAAS blockade. The different relationships between eGFR and RAF in the normo- and micro-albuminuric patients suggest different haemodynamic changes. The reasons for reduced perfusion are unclear.
Supported by: DRWF

1208

Determinants of decline in glomerular filtration rate in association with progression of albuminuria in type 2 diabetes

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Background and aims: Some normoalbuminuric type 2 diabetic subjects have a reduced glomerular filtration rate (GFR), but common and independent risks for GFR decline and albuminuria progression remain unclear.
Materials and methods: An observational 4-year cohort study was performed on 1,002 subjects with preserved GFR (699 normoalbuminuric), and the predictive value of baseline variables on the GFR slope was investigated. GFR decliner and albuminuria progressor were defined as a GFR slope <-4.0 %/year and changes in the geometric mean in urinary albumin from baseline to follow-up >150%, respectively.
Results: Multiple linear regression analysis indicated that GFR slope was predicted by baseline variables of urinary albumin, GFR, HbA_{1C}, systolic blood pressure, plasma total protein, and retinopathy. The effects of these risks appeared variable according to whether individuals had high or low urinary albumin and GFR levels. Subjects cross-classified according to GFR decliner/albuminuria progressor consisted of 51%(-/-), 13%(-/+), 28%(+/-), and 8%(+/+). Common risks for GFR decline and albuminuria progression were retinopathy, neuropathy, HbA_{1C}, and urinary albumin. Independent significant risks for GFR decline were baseline GFR, systolic blood pressure, total protein, and hypertension. Proportion of progression to albuminuria was similar between GFR decliners and non-decliners.

Conclusion: In type 2 diabetes, the GFR slope was predicted and affected at various stages by multiple factors. Isolated GFR decline and albuminuria progression are not rare, and common and independent risk factors predictive for GFR decline and albuminuria progression exist.

1209

The chronic kidney disease collaboration equation: not of additional value compared to the modification of diet in renal disease equation in diabetic patients

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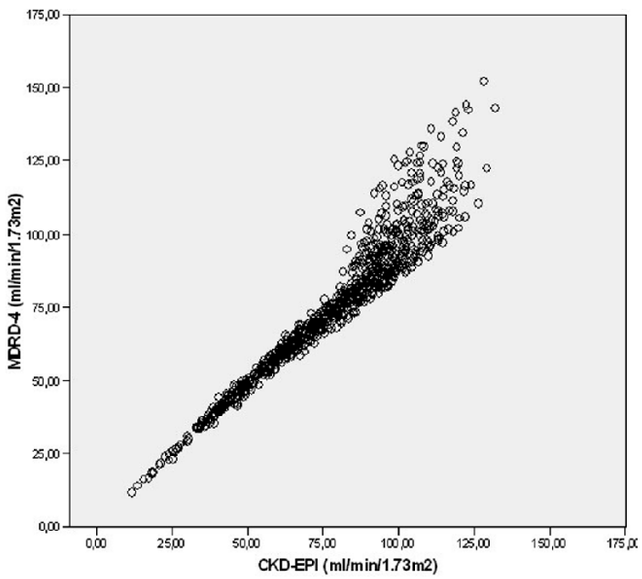
Background and aims: Equations to estimate the glomerular filtration rate (GFR) are routinely used to assess kidney function. Due to the systematic underestimation and limited precision of the current prediction equations, especially when GFR is ≥60ml/min/1.73m^2, a new prediction equation was developed with the aim to reduce these problems: the chronic kidney disease collaboration equation (CKD-EPI). We aimed to compare the outcomes of the CKD-EPI and MDRD-4 equation with the creatinine clearance (CrCl) in a cohort of diabetic adults.
Materials and methods: In this retrospective cohort study of 844 diabetic outpatients, the MDRD-4 and the CKD-EPI were calculated and compared by means of correlation to the 24 hour CrCl, 'the golden standard of daily practice'. MDRD-4 was correlated to CKD-EPI to assess in which clearance ranges differences between the two prediction equations were present. Bias and precision were evaluated to determine the degree of reliability and consistency of both equations. Furthermore, the percentage of subjects having an under- or overestimated kidney function was calculated.



Results: Both the MDRD-4 and the CKD-EPI equation were similarly, though only moderately correlated with CrCl (r=0.69 and r=0.73, respectively). The MDRD-4 showed a high correlation with the CKD-EPI (r=0.98) for patients with an eGFR ≤90ml/min. The correlation decreased to r=0.46 for patients with an eGFR >90ml/min (figure 1). Mean overall bias (SD) of the MDRD-4 and CKD-EPI compared to CrCl were -32.8 (±32.3) ml/min/(1.73m^2) and -33.3 (±32.6) ml/min/(1.73m^2), respectively (p=0.2). Mean bias (SD) between CrCl and MDRD-4 / CKD-EPI in the clearance category >60ml/min were -37.1 (±31.5) ml/min (1.73m^2) / -37.8 (±31.5) ml/min (1.73m^2) respectively (p=0.14). In the different KDOQI stages, the differences between MDRD-4 and CKD-EPI were small and non-significant. When using either CKD-EPI or MDRD-4 for identifying patients with CKD, only approximately 50% of the patients were correctly classified, even when a dispersion of 30% compared to CrCl was accepted.
Conclusion: The CKD-EPI appeared to have no additional value compared to the MDRD-4 when used in a cohort of diabetic patients with a relative preserved renal function. Our data do not support adjustment of current guidelines towards the use of this new prediction equation.

1210

Estimation of glomerular filtration rate in patients with diabetes mellitus type 2: comparison of CKD EPI equation and cystatin C-based formula

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Background and aims: The estimation of Glomerular Filtration Rate (GFR) by the Modification of Diet in Renal Disease (MDRD) equation, which is based on serum creatinine (Scr), has recognised limitations. A new equation was proposed by the Chronic Kidney Disease Epidemiology Collaboration (CKD EPI) to improve estimation of GFR. The CKD-EPI equation showed improved performance compared to the MDRD equation. Moreover, serum cystatin C (ScysC) has been proposed as a potential replacement of Scr in GFR estimation. We compared the CKD-EPI equation to a Scyst based formula for GFR estimation in patients with type 2 diabetes.
Materials and methods: We studied 368 Caucasians participants with type 2 diabetes, 168 (45.7%) men, with [mean (SD)]: age 65 (10) years, BMI 30.7(5.1) Kg/m^2, HbA1c 7.0 (1.5)%. GFR was measured using plasma clearance of 51Cr-EDTA (mGFR). In parallel, GFR was estimated twice, using the CKD-EPI equation [If female and Scr ≤ 0.7 mg/dl, CKD-EPIGFR = $144 \times (Scr/0.7)^{-0.329} \times (0.993)^{Age}$ - if female and Scr >0.7 mg/dl, CKD-EPIGFR = $144 \times (Scr/0.7)^{-1.209} \times (0.993)^{Age}$ - if male and Scr ≤0.9 mg/dl, CKD-EPIGFR = $141 \times (Scr/0.9)^{-0.411} \times (0.993)^{Age}$ - if male and Scr >0.9 mg/dl, CKD-EPIGFR= $141 \times (Scr/0.9)^{-1.209} \times (0.993)^{Age}$] and the Stevens equation which is based on ScystC [cystCGFR: $127.7 / (ScystC^{1.17}) \times (age^{-0.13}) \times (0.91$ if female)]. Estimated GFR results were compared with isotopic GFR by means of two-tailed, paired t tests and by Levene's test for equality of variance. Bland-Altman plots were obtained.
Results: MGFR was 72.0 (22.3) ml/min per 1.73 m^2, CKD-EPIGFR was 83.0 (20.3) ml/min per 1.73 m^2 (p<0.05 for difference from mGFR) and cystCGFR was 72.5 (27.9) ml/min per 1.73 m^2 (NS difference between mGFR and cystCGFR). Bland-Altman plots showed that 95.1% and 93.9% of estimations for CKD-EPIGFR and cystCGFR respectively, lie within the ± 1.96SD of the mean difference. Bias (mean difference between estimated GFR and mGFR) was 10.5 and 0.45 ml/min per 1.73 m^2 for CKD-EPIGFR and cystCGFR respectively (p<0.05 for difference in bias between CKD-EPIGFR and cystCGFR). Precision (SD of the bias) was 13.8 and 21.96 ml/min per 1.73 m^2 for CKD-EPIGFR and cystCGFR respectively (p<0.05 for difference in precision between CKD-EPIGFR and cystCGFR). Accuracy 10% (proportion of estimated GFR results within 10% of mGFR) was 34.8% and 33.2% for CKD-EPIGFR and cystCGFR respectively (NS difference in accuracy 10% between CKD-EPIGFR and cystCGFR). Accuracy 30% (proportion of estimated GFR results within 30% of mGFR) was 72.4% and 72.6% for CKD-EPIGFR and cystCGFR respectively (NS difference in accuracy 30% between CKD-EPIGFR and cystCGFR).
Conclusion: Stevens cystatin C based formula was less biased than and CKD-EPI equation. On the other hand CKD-EPI equation was more precise and presented higher agreement with measured GFR. These results support the superiority of CKD-EPI equation over Stevens cystatin C based formula for estimation of GFR in patients with type 2 diabetes.

PS 120 Nephropathy - biomarkers

1211

Orosomucoid excretion in urine predicts mortality in type 1 and type 2 diabetes

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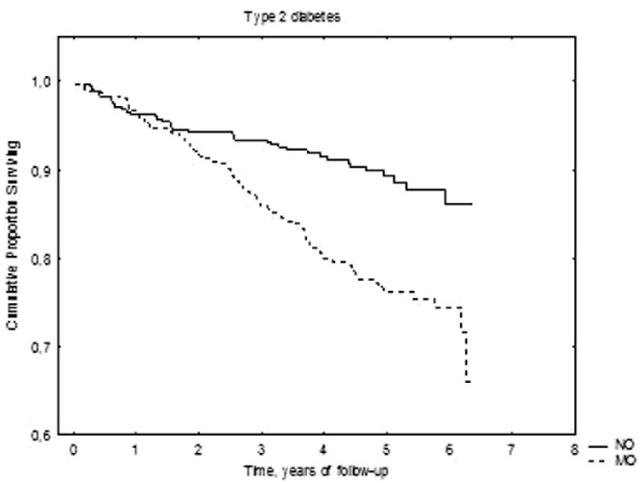
Background and aims: Increased mortality compared to the background population is still a problem of serious concern in diabetes mellitus. Previously we have shown that increased urinary excretion of the inflammatory protein orosomucoid (UOER) independently predicts cardiovascular mortality in patients with type 2 diabetes (T2DM). The evaluation was in a dichotomous comparison. The aim of the present study was to evaluate the predictive value of UOER in a continuous scale on mortality in patients with type 1 (T1DM) and T2DM.
Materials and methods: Patients with diabetes were consecutively included from the outpatient clinic at Amager Hospital provided they had negative urine sticks. Urine samples were analysed for orosomucoid and albumin by immunoturbidimetry. Cut-off value for increased UOER was 2.04 μg/min. Survival analyses were done using Kaplan-Meier survival curves and compared by log-rank test. Cox proportional hazards regression analysis with backward stepwise regression was used for multivariate analysis.
Results: 195 patients with T1DM and 706 patients with T2DM were followed for mean (SD) period of 4.6 (1.3) years. For T1DM mean age was 42 (14) years, HbA1c was 8.6 (1.9) %, median (range) duration of diabetes was 10 (0-44) years and median UOER was 1.00 (0.02-350) μg/min. For T2DM mean age was 59 (11) years, HbA1c was 8.2 (1.8) %, median duration of diabetes was 6 (0-44) years and median UOER was 2.05 (0.03-225) μg/min. Eight patients with T1DM and 120 patients with T2DM died in the follow-up period. In T1DM there was a significant difference in survival between patients with normal (NO) versus increased (MO) UOER ($p<0.006$). There was also a highly significant difference in survival in T2DM ($p<0.0003$) (figure). Using multivariate regression analysis we found that increased UOER (μg/min) (OR: 1.12 (95% CI: 1.04-1.20); $p< 0.003$), age (years) (1.37 (1.27-1.46); $p< 0.00001$) and male sex vs. female (1.12 (1.04-1.20); $p< 0.002$) independently predicted mortality in T2DM. The analyses were adjusted for BMI, HbA1c and systolic blood pressure. When albumin was included in the analysis UOER was not a significant predictor of mortality in T2DM. In a dichotomous comparison UOER was still an independent predictor of mortality in T2DM: NO vs. MO: (1.12 (1.04-1.20); $p< 0.004$), age (1.35 (1.26-1.45); $p< 0.00001$) and sex (1.10 (1.02-1.18); $p< 0.02$) independently predicted mortality. For T1DM: NO vs. MO (1.24 (1.06-1.44; $p< 0.008$)), age (1.18 (1.01-1.37); $p< 0.04$) and BMI (0.83 (0.72-0.97); $p< 0.02$) independently predicted mortality. The latter 2 analyses were adjusted for BMI, HbA1c, systolic blood pressure and microalbuminuria. In a dichotomous comparison microalbuminuria was not an independent predictor of mortality in T1DM or T2DM.
Conclusion: For the first time we have shown that UOER independently predicts mortality in patients with T1DM and confirmed the results in T2DM in a dichotomous comparison. In a continuous scale albuminuria was superior to UOER in T2DM.



1212

Microalbuminuria but not reduced GFR is a marker of subclinical atherosclerosis and arterial stiffness in type 2 diabetes

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Background and aims: Microlbuminuria is a strong predictor for cardiovascular disease in patients with type 2 diabetes. However, the role of reduced renal function, expressed as an estimated Glomerular Filtration Rate (GFR) as a risk assessment tool for macrovascular complications is unclear. The aim of this study was to explore the associations between GFR vs. microulbuminuria and subclinical organ damage in patients with type 2 diabetes.
Materials and methods: Baseline data were analysed from 706 patients who participated in the Cardiovascular Risk factors in Patients with Diabetes - a Prospective study in Primary care (CARDIPP). The Patients, aged 55-65 years, were consecutively recruited 2005-2008 from 22 primary health care centres in Sweden. Urine and blood samples for laboratory analyses were taken in the morning following 10 hours fasting. Presence of microalbuminuria (alb) was defined as u-albumin/creatinine ratio (ACR) >3.0 mg/mmol. GFR was calculated by use of the MDRD formula and reduced renal function defined as < 60 ml/min/1,73m^2. Office blood pressure (BP) was measured by dedicated nurses and 24h-ambulatory BP was also performed. The carotid intima-media thickness (IMT) was determined by ultrasonography. Arterial stiffness was evaluated by pulse wave velocity (PWV) measured as transit time between the carotid and femoral arterial pulse waves. Left ventricular mass was measured echocardiographically, corrected for body surface area expressed as left ventricular mass index (LVMI)
Results: Patients with alb had increased IMT (0.78 vs 0.73 mm), PWV (11.5 vs 10.1 m/s) and LVMI (134.4 vs 118.3 g/m^2) compared to subjects with no alb. The table shows the results further divided and analysed for significance. There were no statistically significant differences in IMT, PWV or LVMI between patients with reduced renal function according to GFR compared to subjects with GFR > 60 ml/min/1.73m^2.
Conclusion: We conclude that microalbuminuria defined as ACR, but not impaired renal function according to GFR, is a marker for subclinical organ damage in terms of atherosclerosis, arterial stiffness and increased left ventricular mass in patients with type 2 diabetes.

Table

	eGFR≥60; alb(-) n=497 Mean ±SD	eGFR<60; alb(-) n=93 Mean ±SD	eGFR≥60; alb(+) n=93 Mean ±SD	eGFR<60; alb(+) n=23 Mean ±SD	P-values eGFR P[#]	P-values urine albumin P[§]
Age (years)	60.5 (3.1)	61.5 (2.7)	60.8 (3.1)	60.5 (3.0)	0.004	0.749
Diabetes duration (years)	6.6 (6.1)	8.0 (6.9)	8.8 (5.5)	9.5 (5.2)	0.015	<0.001
BMI (kg/m^2)	29.9 (4.6)	31.0 (5.6)	30.7 (4.3)	31.4 (3.3)	0.025	0.105
Systolic BP (mmHg)	135 (16)	137 (14)	144 (19)	142 (13)	0.295	<0.001
Diastolic BP (mmHg)	79 (10)	81 (10)	82 (12)	61 (9)	0.480	0.049
HbA1c (%)	5.7 (1.0)	6.0 (1.1)	6.5 (1.2)	6.7 (1.7)	0.415	<0.001
PWV (m/s)	10.2 (2.0)	10.0 (1.8)	11.5 (2.5)	11.0 (1.9)	0.915	<0.001
LVMI (g/m^2)	118.5 (26.8)	119.0 (33.7)	131.5 (32.9)	146.9 (37.4)	0.327	<0.001
IMT (mm)	0.73 (0.18)	0.71 (0.18)	0.79 (0.17)	0.75 (0.26)	0.245	0.009

[#] Differences in means between patients with eGFR < 60 and ≥ 60 analysed with independent samples T test.
[§] Differences in means between patients with urine-albumin/creatinine ratio > 3 and ≤ 3 analysed with independent samples T test.

Supported by: FORSS

1213

Albuminuria is associated with angiographically determined coronary atherosclerosis both in patients with type 2 diabetes and in non-diabetic individuals

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Background and aims: Albuminuria is associated with atherothrombotic events and all-cause mortality in patients with diabetes as well as in non-diabetic individuals. In the present study we aimed at investigating whether albuminuria is associated with directly visualised atherosclerosis in the same manner.

Materials and methods: We enrolled 909 consecutive Caucasian patients, including 226 patients with type 2 diabetes (T2DM) and 683 non-diabetic subjects who were referred to coronary angiography for the evaluation of stable coronary artery disease (CAD). Elevated urinary albumin excretion (UAE) was defined as an urinary albumin to creatinine ratio (ACR) ≥30 μg/mg; significant CAD was diagnosed in the presence of coronary artery lumen narrowing ≥50%.
Results: The prevalence of significant CAD was significantly higher in patients with an elevated UAE than in those with normal UAE (65.9 vs. 51.4%; p <0.001). Logistic regression analysis adjusting for age, gender, smoking, hypertension, LDL cholesterol, HDL cholesterol, CRP, BMI, use of ace/angiotensin II antagonists, aspirin and statins, as well as for the gomerular filtration rate (eGFR) and for T2DM confirmed elevated UAE as a significant predictor of angiographically determined CAD (OR=1.68 [1.15-2.44]; p=0.007). Similarly, the ACR was significantly associated with significant CAD when treated as a continuous variable (standardized adjusted OR=1.45 [1.13-1.86]; p=0.004). The prevalence of elevated UAE was significantly higher in patients with T2DM than in non-diabetic patients (38.9 vs. 18.0%; p <0.001). Like in the total study cohort, the prevalence of significant CAD was higher in patients with elevated UAE than in those with normal UAE out both in patients with diabetes (75.0 vs. 60.9%; p = 0.028) and in those without diabetes (59.3 vs. 49.1; p = 0.040). Concordantly, the ACR proved significantly predictive of significant CAD both in patients with T2DM (1.66 [1.01 - 2.74]; p = 0.045) and in patients without diabetes (1.42 [1.05 - 1.92]; p = 0.023) in a fully adjusted model.
Conclusion: In conclusion, an elevated UAE is strongly associated with angiographically determined coronary atherosclerosis both in patients with T2DM and in non-diabetic patients, independent of conventional cardiovascular risk factors and of the eGFR.

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Seasonal variations of urinary albumin/creatinine ratio in subjects with type 2 diabetes and nephropathy

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Background and aims: It has been recognized that blood pressure shows a seasonal variation. There have been some reports about seasonal change of glycemic control and onset of diabetes. But it remains unknown whether diabetic nephropathy may show a seasonal variation in relation to blood pressure. In the present study, we investigated whether there may be a seasonal change in albumin/creatinine ratio (ACR).
Materials and methods: A total of 430 subjects (male : n=275, female : n=155) with type 2 diabetes and early nephropathy were included, whose mean age was 64.8±0.8 SE years. Those who had advanced stage of nephropathy with increased creatinine were excluded. Data were obtained from 2006 to 2009 at each time of the visit and one-way ANOVA (SPSS-II for windows) was used to determine the presence of seasonal variation.
Results: There were significant seasonal variations in ACR, systolic blood pressure and A1C. The mean ACR was higher in winter (December - February ; 72.8±4.4mg/gCr) than that in summer (June - August ; 54.6±3.4mg/gCr) (p=0.001). The mean systolic blood pressure was higher in winter (136±0.68mmHg) than that in summer (133±0.68mmHg) (p<0.001). The ACR variation showed similar curve to that of the seasonal variation of systolic blood pressure. In contrast, A1C did not show similar curve to that of ACR or systolic blood pressure. The mean A1C was higher in spring (March - May ; 7.39±0.03 %) than that in fall (September - November ; 7.16±0.03 %) (p<0.001). No significant seasonal variation was observed in estimated glomerular filtration rate and diastolic blood pressure.
Conclusion: Our results suggest that there is a hitherto unknown seasonal variation in ACR, and that it may be necessary to consider the seasonal change of ACR especially when we perform intervention study of the nephropathy.

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Factors associating with renal interstitial fibrosis in type 2 diabetics with renal artery stenosis and in type 1 and 2 diabetics with diabetic nepropathy

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Aims: to estimate renal interstitial fibrosis and endothelial dysfunctions factors in type 2 diabetics patients (T2DP) with renal artery stenosis or diabetic nephropathy (DN) and in type 1 diabetics with DN.
Methods: We studied 33 T2DP without renal pathology (I group), 33 T2DP with renal artery stenosis (II group), 24 T2DP with DN (III group) and 30 diabetic type 1 patients with DN (IV group). Patients with T2DP were invited to undergo multispiral computer tomography or selective angiography of renal arteries to define the presence of renal artery stenosis (renal artery stenosis more than 60%). We have measured following parameters in blood: transforming growth factor (TGF - β 1), monocyte chemotactic peptide-1 (MCP-1), regulated on activation normal T-cell expressed and secreted (RANTES), matrix metalloproteinase 9 (MMP - 9),vascular endothelial growth factor (VEGF), vascular cell adhesion molecule (VCAM-1), inhibitor activator of plasminogen (IAP-1), asymmetric dimethylarginine (ADMA), factor von Willebrand (FW), homocystein (HCYST) were measured. The control group included normotensive persons of more than 45 years without diabetes (n=20). Glomerular filtration rate (GFR) was calculated by the MDRD equation.
Results: Studied parameters were higher in groups with renal pathology than in group I (Table 1).

Studied parameters in groups Table 1

Parameter	I group n=33	II group n=33	III group n=24	IV group n=30
TGF - β 1 ng/ml	40,6 [8,6; 81]	121 [11,9; 78]***	80,6 [31,7; 128]**	65 [18,3; 104]***
MMP-9 ng/ml	123 [88,5;149]	141 [102; 176]***	258 [156; 319]***	269[160;362]***
MCP-1 pg/ml	127 [60;175]	198 [59; 262]*	282 [220; 346]	268 [169;345]***
RANTES pg/ml	21972[8747; 32747]	22896[10494; 27193]	28316 [14604; 35758]*	39509 [17882; 72576]**
HCYST mkmol/L	9,8 [7,8; 11,7]	13,5 [8,9; 14,3]*	73,8 [8,3; 75,2]	13,5[8,9;14,3]
FW UE/ml	0,74 [0,191;1,9]	0,9 [0,79; 1,14]***	10,1 [0,2; 1,3]*	0,76[0,13;0,85]***
IAP-1 ng/ml	40,8 [13,9; 55]	61,9 [31,7; 81]*	48,2 [17; 69]	58,9 [25; 87]**
ADMA mkmol/l	1,42 [0,02; 1,46]	14,1 [0,02; 1,4]**	1,7 [0,4; 1,6]	-
VEGF pg/ml	182 [49; 263]	284 [140; 386]**	203 [61,9; 223]	184 [81; 259]

Results are Me[25%;75%]
* - p <0,05 ** - p<0,01 *** - p<0,0001 vs group I

In groups with renal pathology TGF - β 1, MCP-1, RANTES, MMP - 9, HCYST, were negative correlated with GFR and were positive correlated with albuminuria.
Conclusion: DN in type 1 and 2 patients and renal artery stenosis in T2DP were associated with significant increase of mediators of fibrosis and endothelial dysfunctions factors which is known to have a predictive role in the development of the tubulointerstitial expansion and reduction of renal function.

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Urinary proteomics for early diagnosis in diabetic nephropathy

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Background and aims: Diabetic nephropathy may be detectable even at early stages in the urinary proteome. In this study we present recent data indicating that urinary proteome analysis is a valuable tool for early and sensitive

detection of diabetes-associated patho-physiological changes, assessment of disease progression and monitoring of therapy success.
Materials and methods: High-resolution capillary-electrophoresis coupled to time-of-flight mass-spectrometry (CE-MS) was used to profile the low-molecular-weight proteome in urine of diabetic patients collected in longitudinal trials for up to 15 years at two different clinical centers in Denmark and Australia.
Results: When applying previously defined biomarker patterns for chronic kidney disease onto the data obtained from urine samples of normoalbuminuric subjects, we could demonstrate that these biomarkers enabled prediction of development of macroalbuminuria with an AUC=0.92 for a period of 3-4 years. One of the hallmarks of diabetes and associated complications appears to be the increase in extracellular matrix (ECM) and the release of its components, most notably collagen. This process appears to be in part due to reduced proteolysis and is reflected at a very early stage by the decrease in urinary collagen fragments. Assessment of these results in a much higher accuracy of predicting DN than clinical parameters like urinary albumin or serum creatinine.
Conclusion: Urinary proteome analysis enables the non-invasive assessment of diabetic kidney disease at an early stage via determination of specific collagen fragments, opening an avenue towards targeted therapeutic intervention. Further, prognosis of progression towards DN will help targeting therapeutic intervention, ideally before irreversible damage has occurred.

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Adiponectin is differently associated with nephropathy in type 1 and type 2 diabetes

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Background and aims: The aim of this study was to compare the relation between adiponectin and nephropathy in type 1 and type 2 diabetes.
Materials and methods: ApN, C-reactive protein (CRP), fibrinogen (FIB), homocysteine (HCY), lipoprotein(a) [Lp(a)], creatinine clearance (CrCl), creatinine, fasting (fPG) and postprandial plasma glucose (ppPG), glycated haemoglobin (A1c), blood pressure (BP), liver function, lipids, ferritin, uric acid (UA), creatine phosphokinase and leucocyte count (WBC) were determined in 164 patients with type 1 (DM1) and type 2 diabetes (DM2). The patients were assigned to subgroups based on their 24-h albumin excretion rate (AER) [<30 (NA), 30-300 (MI), >300 (MA)] and CrCl (normal >0.83 mL/sec for women and >1.17 mL/sec for men (CrCl1)). Differences between types of DM were tested using Student t test or Mann Whitney test if assumption of homogeneity of variance was not met. Differences between ApN according to AER and CrCl were tested using factorial analysis of variance.
Results: Statistically significant differences were found among ApN values according to AER (F=8,45, df=2, p<0.001) in DM1 (NA=12.37±6.62, MI=21.38±7.98 and MA=31.85±18.05) and DM2 (NA=9.05±5.63, MI=7.46±4.58 and MA=5.26±3.3). A statistically significant difference in ApN between the types of DM (F=73.402, df=1; p<0.001), and an interaction between DM type and AER (F=18.12, df=2; p<0.001) were also observed. DM1 had significantly higher ApN than DM2. Significant within-group differences for ApN were found in DM1 between the NA and MI, and the NA and MA subgroups using Tukey post hoc test, while between-group differences in ApN were found in the MI and MA subgroups of DM1 and DM2. In a model for ApN as a dependent variable and the type of DM, CrCl and interaction DM type and CrCl as factors, a statistically significant difference was found for all analysed factors. ApN was found to be higher in CrCl2 than in CrCl1 (F=12.7, df=1, p<0.001) in both types of diabetes (DM1: CrCl1=13.9±7.93 vs. CrCL2=23±12.8 and DM2: CrCl1=7.63±4.76 vs. CrCl2=9.86±6.25). Post hoc test showed that ApN was significantly increased in both CrCl subgroups of DM1 as compared to DM2. In a model for Lp(a) as a dependent variable and the type of DM (F=0.82; df=1; p=0.37), AER (F=0.21, df=2, p=0.81) and interaction between DM type and AER (F=0.06; df=2; p=0.93) as factors, there were no statistically significant differences. After stepwise regression in DM1 for ApN, the best model (R^2=0.9002) included CrCl, BMI, LDL, WBC, CRP and age, whereas in DM2 the best model (R^2=0.2882) included ppPG, LDL, and UA. In DM1 ApN correlated significantly (p<0.05) with HCY (r=0.57), CrCL (r=-0.61), AER (r=0.61) and creatinine (r=0.40), and in DM2 with HCY (r=0.25), CrCl (r=-0.22), creatinine (r=0.20) and diastolic BP (r=-0.19). ApN and HDL were significantly increased (p<0.001) in DM1, whereas CRP (p=0.04), FIB (p<0.001), HCY (p<0.001) and gamma-glutamyl-transpeptidase (p<0.05) were significantly increased in DM2.
Conclusion: ApN was increased in both DM1 and DM2 in the subgroups with decreased CrCl, but with a different albuminuria-related behaviour, showing an increase with a progression of albuminuria in DM1, and a decrease with a progression of albuminuria in DM2. Other inflammatory markers were decreased in DM1. The interaction between renal insufficiency and albumin loss appears to significantly affect ApN level, which is consistent with different courses of nephropathy in DM1 and DM2.
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The expression of pigment epithelium-derived factors, matrix metalloproteinase-2 and transforming growth factor-β_1 and the effects of rosiglitazone in diabetic rat kidney

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Background and aims: To investigate the expressions of pigment epithelium- derived factor, matrix metalloproteinase-2 and transforming growth factor-β_1 in the kidney of diabetic rats and the effects of rosiglitazone on such changes.
Materials and methods: 45 male SD rats were randomly divided into normal group(NC), diebetic control group(DC) and rosiglitazone treated group(DR), with 15 respectively. After 12 weeks, kidney mass/body mass, 24-hour urinary albumin excretion (UAE), serum creatinine(Scr), blood ureanitrogen (BUN) of every group were measured and the expression of PEDF, MMP-2 and TGF-β_1 in the kidney were determined by immunohisto- chemistry and RT-PCR methods.
Results: (1)KI, BUN, Scr, UAE, TG in DC group were higher than NC group, but decreased in DR group compared with DC group, the differences were statistically significant. (2) Immunohistochemistry showed that renal PEDF, MMP-2 expression in DC and DR group of were lower than NC group (P <0.01), while increased in DR group compared with DC group (P <0.01); the expression of TGF-β1 in DC and DR group were higher than NC group (P <0.01), while TGF-β1 expression in DR group decreased compared with DC group (P <0.01). (3)RT-PCR analysis showed that rosiglitazone can enhance the expression of PEDF mRNA in the kidney, the differences were statistically significant. (4)Correlation analysis showed that there was a negative correlation between the protain expression levels of PEDF or MMP-2 and TGF-β_1 in the kindey of diabetic rats (r=-0.964,P<0.01;r=-0.916,P<0.05). The protein expression levels of PEDF and MMP-2 showed a positive correlation in the kindey of these rats(r = 0.827,P <0.01).
Conclusion: Renoprotection of rosiglitazone on diabetic rats may be mediated through increasing the expression of PEDF and MMP-2 and decreasing the expression of TGF-β_1.

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Abnormalities of the OPG/RANKL axis in progressive kidney failure: the variable influence of diabetes

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Background and aims: The high prevalence of cardiovascular disease and vascular calcification in chronic kidney disease (CKD) is associated with both traditional and uremic-specific risk factors. These include hyperphosphatemia, high calcium x phosphate product, chronic inflammation, dyslipidemia and other dialysis-related factors. Recently the role of osteoprotegerin (OPG) and receptor activator of nuclear factor kappa-β-ligand (RANKL) in bone and vascular adaptations has become apparent. This study was designed to investigate the influence of kidney failure on this axis in diabetic and non diabetic subjects.
Materials and methods: Seventy patients were studied - 20 with Stage 3 CKD (CKD_{Mod}), 20 with Stage 4 and 5 CKD (CKD_{Adv}) and 30 on Haemodialysis (CKD_{HD}). Each groups had equal number of diabetics and non-diabetics. Serum OPG and RANKL were measured along with parameters reflecting renal function and mineral metabolism.
Results: Mean OPG levels in the CKD_{HD} group were higher than in the CKD_{Adv} group (p< 0.001) and the CKD_{Mod} group (p<0.001) (9.8 vs. 5.8 vs 5.4

respectively). There were no differences in RANKL levels across the groups. The OPG/RANKL ratio was significantly different across the groups (p = 0.038). Median OPG/RANKL ratio was higher in the CKD_{HD} group than in the CKD_{Adv} group (p = 0.016) and the CKD_{Mod} group (p = 0.066) (109 vs. 45: vs. 61 respectively). In the CKD_{Mod} group OPG levels were higher in diabetic patients than in non-diabetics (6.1 vs. 4.5 pmol/l: p = 0.032). The same was true in the CKD_{Adv} group (6.7 vs. 4.6 pmol/l: p = 0.032). However, in the CKD_{HD} group, diabetics had lower OPG levels (8.6 vs. 10.9 pmol/l), though this difference did not reach statistical significant (p = 0.079).
Conclusion: OPG is secreted by endothelial cells and tends to protect against VC. Rising OPG levels in progressive kidney failure imply mobilisation of protective adaptations to the increased calcific stimulus generated by falling kidney function. The higher OPG levels in diabetics in moderate to advanced CKD can be similarly interpreted. The reversed ratio in HD patients suggests relative failure of protective mechanisms in diabetics in this setting, resulting in an increased calcification potential.
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PS 121 Nephropathy - treatment

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Blockade of advanced glycation end products receptor signalling prevents the glycated albumin induced overexpression of collagen IV
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Background and aims: The expansion of mesangial cells, accumulation of extracellular matrix protein, thickening of glomerular and tubular basement membranes, tubulointerstitial fibrosis and glomerulosclerosis occur in the diabetic kidney and AGEs play an important role in these pathological changes. AGEs may promote pro-fibrotic cellular responses by interacting with their receptor-RAGE or by other pathways in which TGF-β has a pivotal role. Our aim was to investigate the relationship between AGE, RAGE and TGF-β in collagen IV expression in cultured human embryonic kidney cells (HEK293) exposed to glycated BSA (AGE-BSA).
Materials and methods: Cultured cells were treated for 24 hours with AGE-BSA or BSA (control) at concentrations between 50-200 μg/ml in the presence or absence of 20 ng/ml anti-RAGE antibodies. The level of RAGE, TGF-β1 and procollagen α1 (IV) mRNAs was analyzed by quantitative real-time PCR whereas their protein expression was assessed by Western immunoblot (RAGE and collagen IV) and ELISA (TGF-β1).
Results: The mRNA relative expression ratio (R) increased for all target genes proportionately with AGE-BSA concentration. At 100 μg/ml AGE-BSA, R increased to 1.41+/- 0.12, 1.23+/- 0.05, 2.68+/- 0.17 for RAGE, TGF-β1 and procollagen α1 (IV), whereas at 200 μg/ml AGE-BSA was the highest increase of R to 1.83+/- 0.2, 3.4+/- 0.09 respectively 4+/-0.12. The protein levels of RAGE, TGF-β1 and collagen IV were in good correlation with the mRNA expression. The co-treatment with anti-RAGE antibody and 100μg/ml AGE-BSA versus anti-RAGE antibody and 100μg/ml BSA decreased R to 0.65+/-0.09 for procollagen α1 (IV), whereas the mRNA expression of TGF-β1 remained unchanged. In addition, R increased to 2.1 +/-0.2 for RAGE. The relative proteins levels decreased to 0.42 for collagen IV and to 0.62+/-0.12 for protein TGF-β1 but the RAGE one was 1.6 suggesting that the presence of ligand stimulated RAGE expression.
Conclusion: This study demonstrated that the collagen IV synthesis is modulated by the axis AGE-RAGE-TGF-β1, and was increased in an AGE-BSA concentration dependent manner. It appeared that the presence of AGE-BSA induced the increase of RAGE expression when the number of receptors was diminished by anti-RAGE antibodies treatment. These events were probably involved in the development of fibrosis processes related to diabetic nephropathy.
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Aldosterone blockade ameliorates nephropathy by increasing glucose-6-phosphate dehydrogenase activity and reducing oxidative stress in diabetic hypertensive rats
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Background and aims: Oxidative stress is at the center of pathogenesis of diabetic nephropathy. Hyperglycemia decreases glucose-6-phosphate dehydrogenase (G6PD) activity that makes cells very sensitive to oxidant damage via reducing NADPH formation. Spironolactone, a mineralocorticoid receptor blocker, diminishes hyperglycemia induced reduction in G6PD activity. In the present study we investigated whether spironolactone improves nephropathy by increasing G6PD activity and reducing oxidative stress in hypertensive diabetic rats.
Methods: Spontaneously hypertensive rats were rendered diabetic by intravenous injection of streptozotocin (50 mg/kg), control rats received citrate buffer. The diabetic animals were randomized to receive or not receive spironolactone (50mg/kg/day) for 8 weeks. Albumin excretion rate was determined in 24 h urine by ELISA. Renal expression of fibronectin and p47phx were assessed by Western blot. G6PD activity was determined in renal cortex by estimation of the rate of production of NADPH. The antioxidant system,

glutathione, was estimated as the ratio of reduced form of glutathione (GSH) / oxidized form of glutathione (GSSG) by an enzymatic method. Comparisons between groups were done with one-way analysis of variance (ANOVA) followed by Bonferroni test. Nonparametric data were analyzed by Kruskal-Wallis test (for multiple groups) and Mann-Whitney U test (for 2 groups). A value of $p<0.05$ was considered significant.
Results: Plasma glucose levels were higher ($p<0.0001$) in diabetic rats and it was not modified by spironolactone. Likewise, systolic blood pressure was unaltered by diabetes or by treatment. Albuminuria and renal expression of fibronectin were higher ($p=0.01$ and $p=0.03$, respectively) in the diabetic group compared to control, and these parameters were reduced with the mineralocorticoid receptor blockade. G6PD activity and the GSH / GSSG ratio were reduced ($p=0.008$ and $p=0.02$, respectively) in diabetic rats and the treatment restored to control levels. Urinary levels of 8-hydroxi-deoxiguanosine (8-OHdG), a marker of oxidative stress-induced DNA damage was determined by ELISA and found to be higher ($p=0.02$) in diabetic rats when compared to controls, and the treatment reduced to control levels. The production of superoxide induced by NADPH oxidase (lucigenin) and p47phox, an isoform of NADPH oxidase, was higher ($p=0.009$ and $p=0.004$, respectively) in diabetic rats when compared to controls and was significantly reduced in treated rats.
Conclusion: These results suggest that spironolactone ameliorates nephropathy in the diabetic hypertensive rats by restoring G6PD activity and diminishes oxidative stress without affecting blood pressure.
Supported by: CAPES and FAPESP

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Uncoupled endothelial nitric oxide synthase is ameliorated by green tea (camellia sinensis) in diabetic SHR rats
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Background and aims: It has been shown that in hypertensive diabetic rats green tea (GT, *Camellia sinensis*) ameliorates nephropathy by reducing oxidative stress. In experimental diabetic nephropathy, NADPH oxidase and eNOS uncoupling are major sources of local superoxide production. Uncoupled eNOS is a consequence of a reduction in tetrahydrobiopterin (BH4), an essential cofactor required for the synthesis of nitric oxide (NO). Therefore, uncoupled eNOS leads to a reduction of NO bioavailability which is associated with progression of diabetic nephropathy. The aim of the present study was to investigate if green tea can ameliorates uncoupled eNOS in hypertensive diabetic rats.
Materials and methods: Twelve-week-old spontaneously hypertensive rats (SHR) were rendered diabetic by intravenous injection of streptozotocin (50 mg/Kg), control rats received citrate buffer. Diabetic SHR rats were randomized to receive no treatment or treatment with daily, freshly prepared, GT (13.3 g/L). After 12 weeks of treatment, endothelial and oxidative stress markers were assessed in renal cortex. The levels of BH4 were measured in urine and renal cortex samples using UPLC (Ultra Performance Liquid Chromatography). The results were compared by analysis of variance (ANOVA) followed by Fisher's protected least-significant difference test.
Results: The systolic blood pressure did not differ between groups of the study. However, body weight was less ($p < 0.0001$) and glycemia was greater in diabetic SHR rats (treated or not with GT) than in nondiabetic rats ($p < 0.0001$). Oxidative stress, assessed by NADPH oxidase induced superoxide production, was higher in diabetic rats than in controls ($p=0.01$). The formation of peroxynitrite, a result of the binding of NO to superoxide also increased in diabetic rats ($p = 0.01$). GT attenuated renal ROS production by decreasing the production of superoxide ($p=0.01$) and nitrotyrosine (NT) ($p=0.04$). In diabetic animals, the caveolin-1 (CAV-1), a negative regulator of eNOS expression, was significantly increased ($p=0.02$) and reinstated by GT treatment ($p=0.01$). Immuno-precipitation studies revealed a significant decrease in the CAV1-eNOS binding in diabetic rats ($p=0.03$). Total biopterin, BH4 and oxidation rate of BH4 were measured in urine and renal cortex to assess the bioavailability of NO and the eNOS uncoupling. In diabetic rats, we observed a significant decrease ($p<0.0001$) in the levels of total biopterin and BH4 and moderate change ($p=0.004$) in oxidation rate of BH4 in both urine and renal tissue. GT reversed oxidation of BH4 ($p=0.05$), but not modified BH4 production.
Conclusion: In summary**,** green tea GT ameliorated superoxide production and improved the eNOS uncoupling by reversing CAV-1 expression and oxidation of BH4.
Supported by: CNPq and FAPESP

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Cilnidipine additively inhibits the progression of renal impairment in diabetic rats when used in combination with an angiotensin II receptor blocker (ARB)
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Background and aims: Cilnidipine represents the only calcium channel blocker (CCB) currently available that antagonizes not only the L-type but the N-type calcium channels and is known to provide renal protection by decreasing the activity of the sympathetic nervous system and the renin-angiotensin system (RAS). However, very few studies evaluated cilnidipine for renal protection in diabetic nephropathy. In this study, therefore, we investigated the effect of the L/N-type CCB cilnidipine, as compared to the L-type CCB amlodipine, on diabetic nephropathy in spontaneously type 2 diabetic rats (OLETF rats), when used in combination with an angiotensin II receptor blocker (ARB).
Materials and methods: Seventeen-week-old OLETF rats were randomly assigned to receive cilnidipine (Cil), amlodipine (Aml), valsartan (Val), Cil + Val, Aml + Val, or vehicle (5% HMC; control) for 22 weeks via a gastric tube, with a total of 16 rats assigned to each group.
Results: Antihypertensive potency was found to be nearly equal between the monotherapy groups and between the combination therapy groups compared. Blood pressure lowering with either treatment did not significantly affect the glycemic variables evaluated. However, the increases in urinary albumin excretion (UAE) seen with progression of diabetic renal impairment were significantly suppressed in the rats given Cil or Val, with their additive suppression seen in those given Cil + Val. Furthermore, the norepinephrine (NE) level in the renal tissue as an indicator of sympathetic nervous system activity was shown to be significantly decreased in those given Cil + Val. With regard to the plasma RAS-related variables, Val tended to increase plasma renin activity (PRA) and angiotensin II (Ang II) activity through a feedback mechanism resulting from inhibition of the Ang II type 1 receptor (AT1-R). Additionally, while significant increases in PRA and Ang II were seen in those given Aml + Val, as may be the case with antihypertensive therapy, no increases in PRA and Ang II were seen in those given Cil + Val, a comparably potent antihypertensive regimen.
Conclusion: Study results revealed that cilnidipine produces additive antihypertensive and UAE-lowering effects when combined with an ARB even in type 2 diabetic rats. Furthermore, combination therapy with cilnidipine and valsartan has been shown to significantly reduce NE secretion, suggesting that cilnidipine suppresses the increases in PRA and Ang II associated with antihypertensive therapy by inhibiting the activity of the sympathetic nervous system through its N-type calcium channel antagonism. Thus it is suggested that cilnidipine may inhibit the progression of nephropathy in type 2 diabetes by inhibiting the activity of the sympathetic nervous system as an N-type CCB.

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Telmisartan, an angiotensin II type 1 receptor blocker, prevents renal injury via inhibition of the Notch pathway in Ins2 Akita diabetic mice
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Background and aims: There are an increasing number of patients with end-stage renal disease caused by diabetic nephropathy (DN). It has been recently reported that the Notch pathway is involved in the pathogenesis of DN and the activated Notch pathway induces apoptosis to the podocytes. We investigated the activation of the Notch pathway in Ins2 Akita (Akita mouse), a murine model of DN, and the effects of telmisartan, an angiotensin II type1 receptor blocker, on the Notch pathway and whether telmisartan could prevent podocytes apoptosis.
Materials and methods: Akita mice and control mice received telmisartan (5 mg/kg/day) or no treatment, respectively, for 15 weeks. Body weight, blood pressure, and urinary albumin excretion were measured. The effects of telmisartan on the Notch pathway were studied by RT-PCR and immunohistochemisty both *in vivo* and *in vitro* using cultured murine podocytes. And podocytes were treated with angiotensin II (AII) in the presence or absence

of telmisartan. After that, apoptosis was defined as the presence of nuclear condensation on Hoechst staining. Alternatively, the Annexin V/propidium iodide assay was carried out and analyzed by flow cytometry.
Results: Compared to the control mice, the levels of urinary albumin excretion, serum BUN, and creatinine were higher in the Akita mice (10.9 mg/day, 22.2 ± 3.8 mg/dl, and 0.07 ± 0.01 mg/dl vs. 50 mg/day, 64.7 ± 12.3 mg/dl, and 0.19 mg/dl, respectively; $P < 0.05$). Telmisartan treatment significantly decreased those in Akita mice (33 mg/day, 30.2 ± 6.7 mg/dl, and 0.09 ± 0.01 mg/dl, respectively; $P < 0.05$). The intracellular domain of Notch1 (ICN1) is proteolytically cleaved from the cell membrane in the course of the Notch activation. The expression of ICN1 and its ligand, Jagged1, were increased in the glomeruli of Akita mice, especially in the podocytes. Administration of telmisartan significantly ameliorated the expression of ICN1 and Jagged1. Telmisartan inhibited the AII-induced increased expression of transforming growth factor β (TGF-β) and vascular endothelial growth factor A (VEGF-A) which could directly activate the Notch pathway in cultured murine podocytes. TGF-β and VEGF-A increased the expression of the Notch target gene, Hairy /Enhancer of split-related 1 (*Hes1*), and telmisartan suppressed those expression. Flow cytometer studies showed that apoptotic cells were increased in the podocytes treated with AII (12.56 ± 1.9% vs. 7.09 ± 1.4% in the control group, $P < 0.01$) and telmisartan treatment significantly decreased the AII-induced apoptotic cells (8.51 ± 2.0% vs. 12.56 ± 1.9% in the AII group, $P < 0.01$). We also examined the apoptosis by the use of Hoechst 33342 staining. Nuclear condensations were observed in the podocytes in the presence of AII and those changes were significantly decreased when the podocytes were treated with telmisartan.
Conclusion: The Notch signaling pathway was activated in podocytes in Akita mice. Telmisartan suppressed the Notch pathway both *in vivo* and *in vitro*. And telmisartan suppressed the podocyte apoptosis induced by AII. The AII induced podocytes apoptosis via the activating Notch pathway and telmisartan inhibited that through the inhibition of the Notch pathway. Our results indicate that telmisartan prevents DN through the inhibition of the Notch pathway.

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Tubular damage in type 2 diabetic nephropathy: the effect of ultrahigh doses of irbesartan

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Background and aims: Blockade of the renin-angiotensin-aldosteron system (RAAS) is renoprotective in diabetic kidney disease, and has been shown to affect both the glomerulus and tubules. We aimed to investigate the effect of the angiotensin II receptor blocker irbesartan on the tubular markers: urinary(u) neutrophil gelatinase associated protein(NGAL), kidney injury molecule 1(KIM1) and liver-fatty acid-binding protein(LFABP).
Materials and methods: Substudy of a double-masked, randomized, cross-over study including 52 hypertensive type 2 diabetic patients with microalbuminuria. After 2 months washout of all antihypertensive medication except bendroflumethiazid, patients were treated with irbesartan 300, 600 and 900 mg o.d. for 2 months. Endpoints: 3x24hour(h) urine albumin excretion(UAER), 24h blood pressure, glomerular filtration rate (GFR, ^{51}CrEDTA) and tubular markers measured at baseline and after each treatment period with ELISA (Roche).
Results: Fifty-two patients completed the study(41 male). Age (mean(SD)): 58(10) years and diabetes duration 13(8) years. At baseline, ambulatory blood pressure was 140(11)/ 77(7) mmHg, GFR 101 (24)ml/min/1.73m^2 and UAER [geometric mean (95%CI)] 133 (103-172)mg/24h. As previously reported UAER was significantly more reduced on 900 mg Irbesartan compared to lower doses. Levels of the tubular markers at baseline were: [geometric mean (95%CI)]: u-KIM1 3.6 (2.9-4.5)(pg/ml)/creatinine, u-NGAL 139 (104-187)(pg/ml)/creatinine, and u-LFABP 42 (29-59)(pg/ml)/creatinine. U-NGAL at baseline were tightly related to GFR (R=0.46, p<0.01), whereas u-LFABP and u-KIM 1 were not (p>0.5). U-albumin at baseline was not associated with any of the tubulus markers: NGAL (R=0.08, p=0.6), u-LFABP (R=0.07, p=0.7) or u-KIM1(R=0.03, p=0.8). With increasing doses of irbesartan (300, 600, 900 mg) u-KIM1 was reduced to (geometric mean) 3.1, 3.3 and 3.1 (p=0.07 between 900 mg vs. baseline and no difference between doses). U-NGAL did not change significantly (135, 135 and 138) (p>0.7 compared to baseline, N.S. between doses). U-LFABP did not change during treatment (57.9, 61.8 and 45.1).
Conclusion: Ultrahigh doses of irbesartan treatment reduced levels of the tubular markers u-KIM1 and u-NGAL in type 2 diabetic patients, although not significant. This is in contrast to previous studies in diabetic nephropathy where an ACE inhibitor has reduced markers of tubular damage. More studies with longer follow up are needed to determine the role of tubular markers in monitoring treatment effect and prediction of prognosis in diabetic nephropathy.

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Influence of rosiglitazone on proteinuria and renal haemodynamic in type 2 diabetic patients with overt diabetic nephropathy

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Background and aims: Proteinuria reflects overt glomerular damage and its degree determines the progression of diabetic nephropathy (DN). Recent studies demonstrated an improvement of renal endothelial function and a reduction of microalbuminuria by activation of the PPAR gamma receptor in early stages of DN. The aim of the present study was to investigate the influence of the thiazolidindione rosiglitazone (RSG) on proteinuria and renal endothelial function in overt DN.
Materials and methods: We conducted a double blind placebo (PLC) controlled study in 28 patients (24 men, 4 women, mean age 66.1 ± 9.1 yr) with type 2 diabetes, proteinuria > 300 mg/24 hr (despite the use of ACE-inhibitor or angiotensin receptor blocker) and an estimated glomerular filtration rate (GFR) < 60ml/min. Patients were randomly assigned to RSG (4mg b.i.d.) or matching PLC in addition to their previous antidiabetic medication. GFR and renal plasma flow were measured by inulin- and p-aminohippurate-clearance before and after the blockade of nitric oxide (NO) by intravenous administration of N-monomethyl-L-arginine-acetate (L-NMMA).
Results: During 12 months of follow up there was a significant reduction of proteinuria in the RSG group (2.46 ± 2.3; 1.25 ± 1.2 and 1.6 ± 1.4 g/24 hr at baseline; 6 and 12 months respectively; P<0.05) but not in the PLC group (1.56 ± 1.4; 1.63 ± 1.9 and 1.66 ± 1.9 mg/24 hr at baseline; 6 and 12 months respectively). HbA1c within each treatment group did not change significantly (7.3 ± 0.8 vs. 7.3 ± 1.1 % for PLC and 6.9 ± 0.8 vs. 6.5 ± 0.7 % for RSG; baseline vs. 12 months follow up; respectively). Decline of GFR during the study was equal between RSG and PLC (4 ml/min). RSG increased intrarenal NO bioavailability as indirectly shown by the infusion of L-NMMA. RSG treatment was associated with more adverse events: most common were increase of body weight and development of peripheral edema, however congestive heart failure did not occur.
Conclusion: RSG reduced proteinuria in overt DN and improved intrarenal NO bioavailability. RSG treatment did not deteriorate GFR but was associated with fluid retention.

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Comparison of nifedipine retard and ACE inhibitor with respect to the influence on renal function in hypertensive patients with type 2 diabetes - J-MIND study / reanalysis using eGFR

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Background and aims: It has been described in JSH2009 that strict blood pressure control as well as glycemic control is important for hypertensive patients with diabetes, and the target blood pressure is aimed at lower than 130/80 mmHg. However, although the first-line drug for this disease is ARB or ACE inhibitor, administration of Ca antagonist with strong antihypertensive effect is needed for attaining the target blood pressure. We have conducted the J-MIND (The Japan Multicenter Investigation of Antihypertensive treatment for Nephropathy in Diabetes) study, a 2-year randomized comparative study of nifedipine retard (N group) and enalapril (E group) with respect to the effect on the onset/progression of nephropathy in hypertensive patients with type 2 diabetes, and reported that the renal protection effect indexed by urinary albumin excretion rate was similar in both groups. Since a formula for estimated glomerular filtration rate (e-GFR) suitable for Japanese was newly established currently, the influence on renal function was comparatively studied using eGFR between the 2 groups.

Patients and methods: The subjects of this study were 424 hypertensive patients with type 2 diabetes at the age of lower than 75 years with 140 mmHg or higher baseline systolic blood pressure (SBP), or 90 mmHg or higher diastolic blood pressure (DBP), in whom eGFR could be calculated. Patients were classified based on CKD Stage (eGFR<60, 60-89, >90) (stage 1: 121 cases, stage 2: 199 cases, stage 3: 104 cases), and the 2-year transition of renal function was compared between the 2 groups. The transitions of blood pressure and eGFR were evaluated using Student's t-test.
Results: The mean dose in the N group and the E group was 28.2 ± 11.5 mg/day and 6.4 ± 2.5 mg/day, respectively, and no significant difference was observed in the status of glycemic control between the 2 groups. In both groups, SBP and DBP showed significant decrease during the period after 6 to 24 months ($p<0.001$), whereas SBP showed significant decrease after 6 and 12 months in the N group compared with the E group ($p<0.001$, <0.05) and DBP showed significant decrease after 6 and 24 months in the N group compared with the E group ($p<0.001$, <0.05). No significant fluctuation was observed in eGFR throughout the period of 2 years in all cases in both groups, showing the maintenance of renal function. When the 2 groups were compared with respect to changes of eGFR by CKD stage, continuous increase in eGFR was observed in the stage 3 patients (eGFR<60) during the period after 6-24 months in the N group ($p<0.05$). Whereas in the stage 1 and 2 patients (60<eGFR<90), no significant increase in eGFR was observed in both groups.
Conclusion: The results of this study suggested that long-term administration of nifedipine retard has renal protection effect similar to or higher than ACE inhibitor in terms of eGFR, and the effect is notable in patients with lower eGFR.

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Prevention of microalbuminuria: predictors for a good response to olmesartan treatment (ROADMAP Trial)

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Background and aims: Microalbuminuria (MAU) is an early sign of diabetic nephropathy and increased cardiovascular risk. We investigated whether early treatment with an angiotensin receptor blocker (ARB) in diabetic subjects with normal albumin excretion delays the occurrence of MAU and analysed subgroups that would benefit most from treatment.
Materials and methods: We studied 4,447 subjects with type 2 diabetes and at least one additional cardiovascular risk factor in a randomized, double-blind, multicentre, controlled, and event-driven (MAU) trial. They received either 40 mg olmesartan medoxomil (OM) or placebo (Pb) od. For a median duration of 3.2 years. In both groups, additional antihypertensive treatment (except ACE inhibitors or ARBs) was used to reach the target BP of <130/80 mmHg.
Results: During the double blind period, 178 (8.2%) subjects in the OM group and 210 (9.8 %) subjects in the Pb group developed MAU (HR: 0.770; 95.1% CI: 0.630 to 0.941, p: 0.01). To identify factors influencing the response to OM treatment, explorative post-hoc subgroup analysis using the corresponding median at baseline as a cut-off were performed. This analysis revealed that the treatment effect on time to onset of MAU was better in subjects with a SBP >135 mmHg than with SBP values ≤135 mmHg. A baseline HbA1c ≤7.3% and an eGFR ≤83.79 were predictors for a better response to OM treatment. Furthermore, less than 5% of patients with a baseline UACR ≤4 mg/g developed MAU during the study and the rate was similar between the OM and Pb treated patients. In contrast, 13.9% in the OM group and 18.1% in the placebo group with a baseline UACR >4 mg/g developed MAU (time-to-onset: HR: 0.75, 95% CI: 0.60 to 0.96, p-value= 0.02).
Conclusion: In subjects with type 2 diabetes olmesartan showed a significant 23% risk reduction regarding time to onset of microalbuminuria. Patients with a baseline SBP>135 mmHg, an eGFR ≤83.79, or an UACR >4mg/g benefit most from olmesartan treatment. ClinicalTrials.gov ID no.: NCT00185159.
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PS 122 Cardiovascular risk and assessment

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Benchmarking cardiovascular event rates in type 2 diabetes controlled clinical studies by modification of the UKPDS risk engine

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Background and aims: With the release of the new FDA recommendations for evaluating CV risk in new antidiabetic therapies, models predicting population CV event rates have become critical to the design of clinical trials. In order to successfully demonstrate no increased CV risk, these trials must be adequately powered, with consideration of the appropriate patient demographics and CV history. Although recent outcomes studies have provided evidence of CV safety for specific agents or agent combinations, baseline patient characteristics and underlying CV risk in these populations are not uniform across studies. While the UKPDS risk engine provides a well-accepted basis for estimating CV risk in patients with newly diagnosed disease, the aim of this analysis was to develop a CV risk prediction benchmark for the general diabetes population.
Materials and methods: Summary CV risk data from ADOPT, ADVANCE, VADT, ACCORD, RECORD, PROactive and BARI-2D studies was directly applied to the UKPDS model, and an evaluation/comparison on the prediction of annualized Major Acute Coronary Events (MACE) event rates (myocardial infarction, stroke and CV death) was performed.
Results: The direct prediction of the annualized CV event rate based on the original UKPDS model was inconsistently over-estimated for all studies except for PROactive [predicted event rate vs. observed event rate with mean (SD) = 1.5 (0.26)]. However, when previous CV history is added to the UKPDS model and evaluated across these studies, a more consistent (yet higher) prediction across all studies was generated [Table, observed vs. predicted rate mean (SD) = 2.1 (0.12)].
Conclusion: The modified UKPDS risk model provides a consistent benchmark for CV event rates in clinical study populations more closely resembling a general population of patients with type 2 diabetes.

	ACCORD	ADVANCE	VADT	RECORD	PROactive	ADOPT	BARI-2D
Original UKPDS Model							
Observed annualized MACE event rate (overall)	2.2	2.1	4.2	1.5	3.6	0.8	4.7
Predicted annualized MACE event rate	3.7	3.8	5.4	2.2	3.3	1.6	5.4
Predicted event rate /observed event rate	1.7	1.8	1.3	1.5	0.9	2.0	1.2
Modified UKPDS Model							
Predicted annualized MACE event rate	5.0	5.0	8.8	2.5	6.6	1.6	10.8
Predicted event rate /observed event rate	2.3	2.4	2.1	1.8	1.8	2.0	2.3

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The role of coronary risk assessment in planning and assessing treatment of type 2 diabetes

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Background and aims: Coronary heart disease (CHD) is the major complication in type 2 diabetes. An individual's risk for coronary events is important for planning future treatment. Furthermore information on the changes in coronary risk over time is useful in assessing the effectiveness of interventions. Finally coronary risk assessment can assist explaining potential treatment benefits for patients and thereby increasing treatment adherence. This study assessed initial and at follow-up (two years later) coronary risk in a cohort of type 2 diabetes patients managed in Australian general practice and reviewed in a tertiary teaching hospital. We also reported the risk factors associated with significant reductions in coronary risk.
Materials and methods: Over 1402 type 2 diabetes patients who had both the initial and the follow-up assessment were included in the study. The data on the following variables have been analysed: age, BMI, HBA1c, blood pressure, cholesterol level, self-reported smoking and exercise data. The CHD risk is calculated using the UKPDS risk engine.
Results: At the follow-up assessment after an average of two years, apart from age, the other contributing variables to CHD risk - HbA1c, blood pressure, cholesterol and % of the smoking patients have all improved. The 5 year CHD risk has significantly increased due to the effects of increasing age (Table 1). After removing age effect (i.e. assuming same age as on the initial assessment) the mean 5 year CHD risk at the follow-up assessment is 13.1%, significantly lower than that at the initial assessment. The improvement of the lipids over the period is the single biggest beneficial factor for the CHD risk. The improvement of these risk factors is mostly due to increased use of medication for cholesterol and blood pressure control, oral anti-hyperglycaemic medication and insulin for controlling blood glucose level. Patients with lower initial HbA1c had maintained their CHD risk after adjusting for age while those who had HbA1c >8% had their CHD risk significantly reduced after age adjusted although the risk remained higher than those with lower HbA1c.
Conclusion: The results showed that all major targeted risk factors for diabetes management, including HbA1c, blood pressure, cholesterol levels and smoking, have improved in this cohort of type 2 diabetes patients. The increased CHD risk is solely a function of age increase and the age adjusted 5 year CHD risk is actually reduced. This reflected an overall adequate management of this cohort of diabetes patients while the biggest beneficial effect is from the improvement of lipid profile.

Table 1. The change of patient characteristic and CHD risk (mean and 95% CI or %)

	Initial assessment	Follow up assessment	P
Age	60.2 (11.98)	62.2 (11.96)	<0.01
BMI	31.4 (6.75)	31.3 (6.76)	<0.05
HbA1c	7.45 (1.55)	7.42 (1.53)	<0.05
Systolic BP	139.2 (21.21)	137.9 (20.56)	<0.05
Diastolic BP	79.0 (11.91)	77.3 (11.63)	<0.01
Total Cholesterol	4.77 (1.04)	4.57 (1.08)	<0.01
HDL cholesterol	1.18 (0.31)	1.22 (0.33)	<0.05
LDL cholesterol	2.73 (0.90)	2.48 (0.90)	<0.01
Smoking %	15.1%	11.9%	<0.01
Medication- none	43.4%	29.4%	<0.01
other medication	47.8%	52.6%	<0.01
insulin	8.8%	16.0%	<0.01
5 year CHD risk	14.6% (14.5)	16.4% (14.88)	<0.01
initial HbA1c			
<7%	9.7% (8.9)	12.4% (11.4)	<0.01
7-8%	14.6% (13.1)	17.4% (14.9)	<0.01
>8%	22.6% (18.8)	22.1% (17.9)	NS

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Residual cardiovascular risk due to persistent dyslipidaemia in statin-treated patients with diabetes mellitus in Ireland: results of the Dyslipidaemia International Study

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Background and aims: Dyslipidaemia is an established independent risk factor for cardiovascular disease (CVD) in patients with diabetes mellitus (DM). This study examined the number of patients with DM on treatment with a statin that had normal and abnormal lipid levels according to ESC/EASD guidelines.
Materials and methods: The Dyslipidaemia International Study (DYSIS) was a multi-national cross-sectional study. In Ireland, patients were recruited consecutively by 58 general practitioners and 4 cardiologists. Entry requirements included age >45 years, statin-treatment for >3 months, consent to physical examination, and at least one lipid profile in the past 6-12 months.
Results: In Ireland 980 eligible patients were studied. 181 patients (20.1%) had DM. Despite patients with DM having significantly lower LDL-c levels than patients without DM, triglyceride and HDL-c levels were less likely to be normal in patients with DM (Table 1). The Odds Ratio (O.R.) for a parental history of DM was 3.32 in the DM group versus the non-DM group. Significant differences in systolic blood pressure levels and anti-hypertensive agents used existed between the groups.
Conclusions: The results of DYSIS in Ireland show that triglyceride and HDL-c levels remain abnormal in statin-treated patients with DM. This is in keeping with the international findings in this study. These patients remain at increased CVD risk and supplementary treatment may be indicated.

Table 1. Biochemical and historical results for DYSIS in Ireland

	Patients with DM N=181 (20.1%)	**Patients without DM** N=719 (79.9%)
LDL-c (mmol/L) [Median and Quartiles]	1.9 (1.4 - 2.3)	2.3 (1.8 - 3.0)*
LDL-c <2.59mmol/L	82.4% (145/176)	62.1% (428/689)*
Triglycerides <1.7mmol/L	53.3% (96/180)	68.3% (477/698)**
HDL-c >1.03 Male/ 1.29 Female (mmol/L)	44.9%	67.1%*
Systolic B.P. (mmHg) [Mean and S.D.]	136.9 (+/- 17.9)	133.9 (+/- 17.4) ***
ACE Inhibitor treatment	59.7%	31.3%* O.R.=3.25
HbA1c (available in 150/181 - 82.9%)	6.9 (6.2 - 8.0)	-
Anti-diabetic therapy	77.3% (140/181)	-
Parental history DM	23.2% (42/181)	8.3%* (60/719) O.R.=3.32

*p<0.0001; **p<0.001; ***p <0.05
EASD/ESC Guidelines 2007: LDL-c target = 1.8 - 2.0mmol/L if DM and CVD; Increased risk of CVD if triglycerides >1.7mmol/L, HDL<1 Male/<1.2 Female

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Factors predicting cardiovascular events in statin-treated diabetic and non-diabetic coronary patients: a prospective cohort study

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Background and aims: Statins are a cornerstone in the management of high risk patients. However, residual risk in patients with established coronary artery disease (CAD) remains high. We aimed at identifying which lipid factors drive vascular risk in statin treated patients with CAD.
Materials and methods: We recorded vascular events over a mean period of 7.2 years in 491 consecutive statin-treated patients with angiographically proven stable CAD, covering 3518 patient-years.
Results: In the total population, low HDL cholesterol (standardized adjusted HR 0.80 [0.67-0.94]; p=0.009), low apolipoprotein A1 (0.84 [0.72-0.98];

p=0.022), a small LDL particle diameter (0.84 [0.72-0.98]; p=0.023), and high triglycerides (1.18 [1.04-1.35]; p=0.013) predicted vascular events, but not total cholesterol, LDL cholesterol, or apolipoprotein B. Factor analysis in the lipid profiles of our patients revealed an HDL-related factor and an LDL-related factor. Concordant with the results for individual lipid parameters, the HDL-related factor (0.76 [0.65-0.90]; p = 0.001) but not the LDL-related factor (p=0.644) predicted vascular events. Patients with type 2 diabetes (T2DM; n=116) were at a higher vascular risk than non-diabetic subjects (52.6% vs. 36.8%; p = 0.002), and like in the total population the HDL-related factor (0.63 [0.49-0.81]; p<0.001) but not the LDL-related factor (p=0.976) predicted vascular risk in diabetic patients.
Conclusion: The pattern of low HDL cholesterol, low apolipoprotein A1, small LDL particles, and high triglycerides drives vascular risk in statin-treated coronary patients, particularly in those with T2DM.

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Cardiovascular disease risk communication for patients with type 2 diabetes: The @RISK Study
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Patients with type 2 diabetes (T2DM) underestimate their risk to develop severe complications, and they do not understand the risk communication of caregivers. According to Leventhal's Self-regulation Theory, patients are not willing to change their lifestyle if they are not informed why this is important. People have correct or incorrect perceptions concerning their disease which determine how they manage their risks to develop severe complications. Providing understandable information on the disease by means of risk communication may change illness perceptions. The aim is to investigate the effects of an intervention focussed on the communication of the absolute 10-year risk to develop CVD on risk perception and illness perceptions in patients with T2DM.
Materials and methods: A randomised controlled trial was performed with newly referred T2DM patients to the Diabetes Care System West-Friesland, a managed care system in the Netherlands. The intervention group (n=131) received CVD risk communication, consisting of an explanation on the causes and consequences of CVD, and possibilities for prevention on top of standard managed care of the DCS. The 10 year risk of developing CVD was explained in natural frequencies and visualised by a population diagram. Controls (n=130) received standard managed care. Outcome measures were appropriateness of risk perception and illness perceptions assessed at baseline, at 2 weeks (immediately after the intervention or control visit), and 12 weeks. Risk perception was measured by asking 'How would you rate your risk of developing CVD in the next 10 years?' The absolute difference between risk perception and the actual CVD risk on the UKPDS risk engine was calculated. The Brief Illness Perception Questionnaire was used to assess illness perceptions. An overall score was calculated by summarizing scores on the 8 items, measured on a 10-point Likert scale. A higher score indicates a more threatening view of the illness. Differences in changes between time points were analysed by t-tests in the intervention and control group.
Results: Mean age was 58.4 ± 10.3 years, the median of diabetes duration was 0.36 (IQR 0.1 - 1.4) years, HbA1c was 6.7 ± 1.3% and 57% were men. In the intervention group, the difference between the actual CVD risk and the risk perception improved significantly between baseline and 2 weeks (see Table 1). This effect remained at 12 weeks. In the control group, no changes were found. No effects were found on illness perceptions.
Conclusion: This innovative risk communication method improved patients' risk perception and this effect remained at the long term. The hypothesis that patients might be better able to manage their disease was not supported, as their illness perceptions showed no changes. Table. Differences in risk perception and illness perceptions in the intervention and control groups.

	Baseline	2 weeks	12 weeks	p (baseline - 2 weeks)	p (baseline - 12 weeks)
Risk perception (difference between risk perception and CVD risk)					
Intervention	9.7 (4.7 - 18.5)	4.6 (1.9 - 11.5)	5.8 (1.8 - 12.1)	0.00 *	0.00 *
Control	8.9 (4.9 - 13.5)	8.9 (5.0 - 14.7)	8.0 (3.6 - 16.3)	0.79	0.22
Illness perceptions					
Intervention	31.7 (12.0)	31.0 (11.4)	30.5 (11.5)	0.54	0.85
Control	31.9 (11.2)	32.0 (12.5)	32.3 (12.4)	0.75	0.55

Data are means (± SD) or median (interquartile range). * P < 0.05
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Characteristics, complications and management of a large multiethnic cohort of younger adults with type 2 diabetes
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Background and aims: An increasing number of young people are diagnosed with type 2 diabetes (T2DM), leading to a high lifetime risk for developing diabetes-related complications. Whether this cohort receives appropriate risk factor management in relation to older T2DM subjects remains uncertain. Our aim is to describe the characteristics and management of a multiethnic cohort of people with T2DM <40 years (<40 cohort) and to compare with T2DM subjects aged ≥ 40 years (≥ 40 cohort).
Petients and methods: Retrospective analysis of data extracted from the last clinic visit of 648 adults (< 40 cohort) attending 2 specialist diabetes centres (A and B) in the UK and a ≥ 40 cohort of 3582 T2DM subjects coming from the specialist centre A. In the < 40 cohort, differences between the first (≤22) vs. fifth quintile (≥33) of age of diagnosis were analysed.
Results: Characteristics of the <40 cohort: 57.9% female, 54.5% Caucasian, 45.5% Black or Minority Ethnic origin (BME, 91.9% from South Asian origin). Median age at diagnosis was 28 years (24-31). Data were extracted after a median diabetes duration of 4.0 years (1.9-7.0). The median BMI was 33.0 kg/m^2 (28.3-38.7), higher in Caucasians (35.0 vs. 30.9 in BME, p<0.0001) and women (34.0 vs. 31.9 in men, p=0.003). Median HbA1c was 8.2% (6.8-9.9) with an HbA1c > 7% in 70%. Cardiovascular risk factors were frequent: 71.8% total cholesterol > 4mmol/l, 54.9% triglycerides > 1.7mmol/l, 45% hypertension. Microvascular complications were also prevalent: 19.8% retinopathy, 14.6% abnormal foot exam, 24.0% microalbuminuria (only available for centre A). Oral antidiabetic drugs were used in 71.6%, insulin alone in 18.6% and both in 24.7%. Insulin was more often used in Caucasians (49.4% vs. 36.2% in BME, p=0.001). 27.7% received antihypertensives, 31.5% a statin and 13.9% aspirin. Women were less likely to be treated for hypertension (22.7% vs. 34.8%, p<0.0001) and dyslipidaemia (22.1% vs. 45.1%, p<0.0001) than men. The first quintile of age of diagnosis had more often retinopathy (22.1% vs. 16.9%, p=0.021) and was treated less aggressively compared with the fifth quintile. Fewer were on insulin (45.6% vs. 46.4%, p=0.039), many were managed with diet only (9.6% vs. 6.2%, p=0.005) and they were less likely to be treated for hypertension (23.2% vs. 32.3%, p=0.583) and dyslipidaemia (30.4% vs. 39.2%, p=0.495). Data were generally comparable between the 2 centres, except for a higher proportion of BME (50.6% vs. 36.1%, p<0.0001) and women (61.3% vs. 51.5%, p=0.009) and lower median HbA1c (8.1% vs. 8.7%, p=0.012) in centre A compared to centre B. Compared to the ≥ 40 cohort, patients in the < 40 cohort were more often female (57.9 vs. 46.1, p<0.0001) and of BME origin (45.5 vs. 30.2 , p<0.0001), had a higher median BMI (33.0 vs. 30.4, P<0.0001) and a higher median HbA1c (8.2 vs. 7.5, p<0.0001).
Conclusion: The <40 cohort represents a more extreme phenotype compared to the ≥ 40 cohort with a high prevalence of inadequately treated risk factors. In particular, patients from the first quintile of age of diagnosis were less aggressively treated. There is a need for tailored strategies to manage this high-risk group.

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LDL-cholesterol is not the best blood lipid predictor of CHD risk in type 2 diabetes
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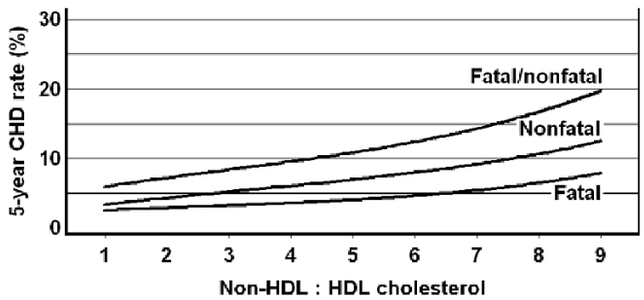
Background and aims: Although frequently used in clinical practice, the validity of LDL-cholesterol (LDL) as a risk factor for coronary heart disease (CHD) is uncertain. We assessed the roles of different measures of blood lipids in an observational study of type 2 diabetes from the Swedish National Diabetes Register (NDR).
Material and methods: 23,001 patients aged 30-75 years, 16% with previous CVD, 43% with lipid-lowering drugs, were followed from 2003 to 2007, with 1709 fatal/nonfatal CHD events. LDL was calculated with Friedewald's equation.

Results: Adjusted hazard ratios (HR) at Cox regression for fatal/nonfatal CHD per 1 SD of each lipid measure were 1.21 (1.15-1.26) with non-HDL/HDL, 1.19 (1.13-1.24) with LDL/HDL, 1.18 (1.13-12.4) with non-HDL, and 1.14 (1.09-1.19) with LDL; all p<0.001 when adjusted for age, sex, diabetes duration, HbA1c, type of hypoglycaemic treatment, systolic blood pressure, antihypertensive drug use, smoking, BMI, and microalbuminuria (>20 µg/min). Goodness-of-fit with global likelihood ratio X^2 values were 771, 762, 757, 739, respectively. Figure 1 shows splines for adjusted 5-year CHD rates as a cubic function of lipids in a Cox model. CHD rates increased progressively with higher non-HDL/HDL ratio as well as non-HDL. When 7889 patients with a combination of non-HDL/HDL >2.9, non-HDL >3.6, and LDL >2.8 mmol/l was used as reference (median values chosen as limits), fully adjusted HR for CHD risk was 0.61 (0.53-0.70) with non-HDL/HDL <2.3 mmol/l (recently often used target; n=6116), HR was 0.67 (0.60-0.76) with non-HDL <3.3 mmol/l (recent target, suggested useful if higher triglycerides; n=7623), while HR was higher 0.74 (0.66-0.83) with LDL <2.5 mmol/l (recent target in guidelines; n=7166). HR with LDL <1.9 mmol/l was 0.65 (0.54-0.80) (suggested target in high-risk patients; n=1889). All HR were p<0.001.
Conclusion: Non-HDL/HDL and non-HDL, which both are reliably measured in the non-fasting state, were more strongly associated with risk of CHD than LDL. Risk reductions for CHD were larger with targets presented here for the non-HDL/HDL ratio and non-HDL, than for LDL. Splines of CHD rate by non-HDL/HDL and non-HDL values underline the "the lower the better" concept for these lipids and CHD risk in type 2 diabetes.

Figure 1: Coronary heart disease (CHD)



Supported by: The Swedish Association of Local Authorities and Regions

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Silent myocardial ischaemia and prediabetes in combination are associated with adverse prognosis in healthy subjects

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Background and aims: Silent myocardial ischemia (SMI) is prognostic for deaths and myocardial infarction. Prediabetes increases risk of diabetes and cardiovascular disease. It was hypothesized, therefore, that prediabetes deteriorates prognosis in persons with SMI.
Materials and methods: Five-hundred-and-ninety-six non-diabetic subjects from the community of age 55 to 75 years and free of any known cardiovascular disease and cancer were examined by ambulant 48h continuous electrocardiogram monitoring. SMI was defined as at least 1 mm depression of the ST interval on the electrocardiogram of at least 1 min duration. Prediabetes was defined as fasting plasma glucose ≥ 5.6 but < 7.0 mmol/L. During a median follow-up of 6.3 years, 77 subjects met the predefined combined endpoint of acute myocardial infarction and/or death.
Results: Two-hundred-and-twenty-nine subjects had prediabetes (38%), which was not associated with SMI (P=0.69). Subjects with prediabetes and SMI (5% of subjects) more often met the combined endpoint (36%) than subjects with prediabetes and non-SMI (15%), subjects with normal fasting glucose (NFG) and SMI (12%), and subjects with NFG and non-SMI (10%), respectively, (P<0.001). Both in a univariate analysis and in a Cox multivariate analysis, the latter of which included the four study groups of interest, and in addition, gender, age, smoking habits, blood pressure and total cholesterol, respectively, only subjects with combined prediabetes and SMI exhibited an increased risk for meeting the predefined endpoint (hazard ratio, HR: 4.0 (2.0-8.1), P<0,001 and HR: 2.5, CI95% 1.2-5.2, P=0.016, respectively; subjects with combined NFG and non-SMI as reference). Including also high sensitive C-reactive protein and NT pro-brain natriuretic peptide in the Cox multivariate model, subjects with prediabetes and SMI exhibited more than a 3-fold increased risk of meeting endpoint compared with reference subjects (HR: 3.2, CI95% 1.5-6.7, P<0.005).
Conclusion: Combined silent myocardial ischemia and prediabetes suggest increased risk of myocardial infarction and/or death among apparently healthy subjects living in the community, thus, this clinical entity calls for screening and treatment.

Fold risk of meeting endpoint of acute myocardial infarction and/or death

	Univariate	Multivariate (a)	Multivariate (b)
NFG and non-SMI (reference group)	1	1	1
Prediabetes (IFG) and SMI	**3.98 (1.95–8.13)*****	**2.50 (1.20–5.20)***	**3.15 (1.49–6.65)****
Prediabetes (IFG) and non-SMI	1.56 (0.94–2.57)	1.42 (0.85–2.36)	1.58 (0.95–2.63)
NFG and SMI	1.32 (0.51–3.40)	1.10 (0.42–2.85)	1.15 (0.44–3.01)

Cox proportional hazard models of combined normal fasting plasma glucose (NFG, </= 5.5 mmol/L)) or impaired fasting glucose (IFG, range: 5.6–6.9 mmol/L) and silent myocardial ischemia (SMI) or non-SMI in relation to the endpoint acute myocardial infarction and/or death.
Hazards ratios (95% confidence intervals); * P<0.02; ** P<0.005; *** P<0.001
(a) Adjusted for smoking, total cholesterol, systolic blood pressure, gender and age.
(b) Additional adjustment for NT-proBNP and high-sensitive CRP, including adjustments made in (a).

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Incident myocardial infarction is five-fold higher in subjects at high risk for type 2 diabetes

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Background and aims: A simple and accurate test comprised of circulating biomarkers (Diabetes Risk Score, DRS) provides a quantitative estimate of the 5-year risk of developing type 2 diabetes mellitus (T2DM). It is established that T2DM is a major risk factor for cardiovascular disease, and that a significant portion of that risk occurs in the prediabetic state. A model using the circulating biomarkers composing the DRS was applied to the Inter99 study to assess the 5-year risk of cardiovascular events (CVE) in groups of subjects at low, moderate and high risk for T2DM.
Materials and methods: Serum samples collected at baseline from 5452 subjects free of diabetes were tested. 2924 (53.6%), 1975 (36.2%) and 553 (10.1%) subjects were classified as low, moderate and high risk for T2DM, with 5 yr T2DM conversion rates of 0.5%, 3.1% and 15.2%, respectively. Relative risk (RR) estimates for overall CVEs and for each of the 4 classes of CVEs (myocardial infarction (MI), re-vascularization (RV), angina (ANG), and stroke (STR)) were calculated as ratios of moderate:low and high:low. P values and confidence intervals were calculated by bootstrap estimation.
Results: The results are summarized in the table below. Relative risks for overall CVEs and all classes of CVEs were significant with the exception of STR.
Conclusion: Several studies have demonstrated that risk of MI is 3-fold higher in diabetics compared to non-diabetics. The data shown here demonstrate similar levels of cardiovascular risk stratification among non-diabetics using the DRS, suggesting that cardiovascular risk factors for patients with a high DRS should be managed carefully.

Relative Risks for Overall Cardiovascular Events by Diabetes Risk Score

	CVE (n=108)	MI (n=42)	RV (n=42)	ANG (n=27)	STR (n=41)
Low DRS	1	1	1	1	1
Moderate DRS	2.7 (p<0.001)	2.6 (p=0.013)	4.1 (p<0.001)	6.5 (p<0.001)	1.8 (p<0.11)
High DRS	3.6 (p<0.001)	4.8 (p=0.003)	6.0 (p=0.004)	6.7 (p=0.03)	2.6 (p=0.09)

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Assessing the influence of modelling subsequent cardiovascular events into a type 2 diabetes cost-effectiveness model

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Background and aims: Type 2 diabetes mellitus (T2DM) is associated with increased risk of cardiovascular morbidity and mortality. Assessing the cost-effectiveness of risk factor modification is commonly based on the analysis of avoiding primary events. The aim of this study was to calibrate published equations to allow the prediction of primary and subsequent events and to assess the economic implications of this within a cost-effectiveness model.
Materials and methods: Routine hospital data from across the UK were analysed between 2000 and 2005 to identify patients with T2DM with first, second and third myocardial infarction (MI) or stroke admissions. The ratio of events (primary + subsequent) to primary event was used to calibrate the cardiovascular risk equations using a published diabetes prevalence-based model. The impact of the calibrated equations was then assessed using a published cost-effectiveness model assessing two treatment strategies: 1st line metformin (a) and (b); 2nd line sulphonylurea add-on (a) versus DPP-4 inhibitor add-on (b); 3rd line thiazolidinedione add-on (a) versus DPP-4 inhibitor add-on (b). The model was run using a UKPDS demographic and risk factor profile with a payer perspective for 40 years with costs and benefits discounted at 3.5%.
Results: Between 2000 and 2005, 1,124,846 T2DM patients were identified, of whom 55,868 and 65,436 experienced a primary MI and stroke, respectively. There were 2,159 (3.86%) and 185 (0.003%) second and third MI admissions, respectively, and 5,808 (8.88%) and 755 (0.012%) second and third stroke admissions, respectively. Incorporating subsequent events into the model had little impact on the cost per quality-adjusted-life-years (QALYs), which ranged from £3,105 to £3,129 with and without subsequent events allowed, respectively. The impact on cost per life-year gained (LYG) was more noticeable; £257,902 with primary events only, and £90,055 with primary and subsequent events.
Conclusion: The inclusion of subsequent cardiovascular events into diabetes models provides greater face validity; however, this has little impact on cost-effectiveness. This study supports the conclusion that the economic assessment of therapies that modify cardiovascular risk factors but do not incorporate subsequent MI and stroke events are not significantly biased - due to the relatively small number of subsequent events. Importantly, this does not imply that treatment in clinical practice should be stopped after first event. Where concerns are raised regarding the suitability and generalisability of risk equations in general, and the UKPDS equations in particular, research should focus on deriving and/or validating primary event risk equations to ensure that the assumption of generalisability is indeed robust. For a general cohort of T2DM patients this would appear to be more pressing than the accurate prediction of subsequent events.

PS 123 Biomarkers and cardiovascular disease

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Effects of six years intensified multifactorial treatment versus usual care on levels of hs-CRP and adiponectin in patients with screen-detected type 2 diabetes. The ADDITION Netherlands study

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Background and aims: Biomarkers as high-sensitivity C-reactive protein (hs-CRP) and adiponectin are associated with cardiovascular disease. Hs-CRP may be a mediator of atherosclerosis. Elevated levels of hs-CRP and decreased levels of adiponectin are independent markers of cardiovascular disease. It is unknown to what extent these biomarkers are influenced by the intensity of a multifactorial therapy aimed at reduction of cardiovascular disease in type 2 diabetes. We compared the effects of six years intensified multifactorial therapy or usual care on levels of hs-CRP and adiponectin in 498 screen-detected type 2 diabetes patients and the association with cardiovascular morbidity and mortality.
Materials and methods: This study is embedded in the Dutch part of the Anglo-Danish-Dutch ADDITION study, a cluster-randomised clinical trial that studies the effectiveness of intensified multifactorial treatment compared to usual care on cardiovascular morbidity and mortality in screen-detected type 2 diabetes patients. Baseline serum levels of hs-CRP and adiponectin were measured at time of diagnosis of type 2 diabetes by screening (2002-2004). Serum hs-CRP was determined by chemiluminescent enzyme immunoassay. Adiponectin was assessed by immunoassay technique. Final measurements were performed in the second half of 2009. In the stored serum hs-CRP and adiponectin will be determined. We will analyse the difference in change in serum hs-CRP and adiponectin levels between both treatment groups with multilevel modelling, to be able to adjust for clustering at the level of the general practice.
Results: Baseline characteristics in both treatment groups (usual care vs. intensive treatment) are comparable, except for the levels of adiponectin (Table). Hs-CRP was 6.9 mg/dl (SD 8.6) in the usual care group vs. 7.3 (SD 9.9) in the intensified treatment group; adiponectin 6759 ng/ml x100 (SD 3845) in the usual care group vs. 5868 (SD 3172) in the intensified treatment group (p=0.006). A total of 330 (66%) patients from 79 general practices participated in the final measurement. Their data are currently analysed and will be presented at the EASD meeting.
Conclusion: This more than 5 years follow-up of multifactorial treatment in screen-detected type 2 diabetes patients will demonstrate whether intensified therapy leads to a significant change in hs-CRP and adiponectin levels and whether such a change is associated with cardiovascular mortality and morbidity.

Baseline characteristics of the ADDITION Netherlands study

Variables	**Usual care group** (n=243)	**Intensive treatment group** (n=255)
Age (years)	59.9 ± 5.1	60.1 ± 5.4
Gender (% male)	56.0	51.8
BMI (kg/m^2)	30.4 ± 4.6	31.2 ± 5.1
Systolic blood pressure (mmHg)	163 ± 23	166 ± 23
Diastolic blood pressure (mmHg)	89 ± 10	90 ± 11
Fasting blood glucose (mmol/l)	8.1 ± 2.8	7.8 ± 2.3
HbA1c (%)	7.4 ± 1.7	7.3 ± 1.6
Cholesterol (mmol/l)	5.6 ± 1.1	5.6 ± 1.1
Hs-CRP (mg/l)	6.9 ± 8.6	7.3 ± 9.9
Adiponectin (ng/ml x100)	6759 ± 3845	5868 ± 3172

Data are percentages or means ± SD

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The ENPP1 K121Q polymorphism predicts accelerated cardiovascular events in obese patients with type 2 diabetes

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Type 2 diabetes is characterized by insulin resistance and accelerated atherosclerosis. The *ENPP1* K121Q polymorphism has been associated with both of these traits, especially among obese individuals. We investigated the role of the *ENPP1* K121Q polymorphism - separately in non-obese and obese individuals (body mass index < or ≥ 30 kg/m^2) - as a predictor of major cardiovascular events in a prospective study of 330 type 2 diabetic patients with coronary artery disease at baseline. Study subjects were followed for 37.1±19.4 months, during which 43 major cardiovascular events occurred (32 cardiovascular deaths, 3 non-fatal myocardial infarctions, and 8 non-fatal strokes). Carriers of the Q121 variant (either KQ or QQ individuals) had an increased risk of incident events among obese subjects (n=159, HR=3.56, 95% CI=1.21-10.46, p=0.02), but not among non-obese individuals (n=171, HR=0.90, 95% CI=0.39-2.06, p=0.81). A similar pattern of association was observed in a cross-sectional study of 339 type 2 diabetic patients (169 subjects from Italy and 170 from the US) who had survived a myocardial infarction. In this analysis, patients who had had the myocardial infarction at a younger age (≤ 50 years, n=112) were compared to those who had had the myocardial infarction at an older age (n=237). Since no genotype-by-sample interaction was observed, data from Italy and the US were pooled and analyzed together. As seen in the prospective study, the Q121 variant was associated with a significantly increase in the risk of early myocardial infarction among obese subjects (n=188; OR=2.51, 95% CI=1.29-4.88, p=0.007), but not among non-obese individuals (n=151; OR, 95% CI=1.11, 0.51-2.42 p=0.878). In conclusion, among obese individuals with type 2 diabetes, the *ENPP1* Q121 variant contributes in accelerating cardiovascular events.

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Leptin and glucose intolerance predict independently a first-ever myocardial infarction with a sex difference - data from a large prospective population-based study in northern Sweden

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Background and aims: The adipocyte-derived hormone leptin mediates several processes associated with glucose hemostasis and atherosclerosis, and data suggests that high leptin may predict diabetes and cardiovascular disease (CVD). As circulating levels and signalling differ between males and females we explored whether leptin also predict first-ever myocardial infarction or sudden death with a sex difference, independent of glucose intolerance.
Materials and methods: This is a prospective nested case-referent study. Subjects (n=564, 40% females) with a first-ever acute myocardial infarction (MI) (fatal and non-fatal, and classified according to WHO) that had participated in population-based studies (the Väserbotten project, the MONICA survey, and the mammary screening cohort) prior to the event (3.9 years) were identified in the Northern Sweden MI registry. Matched (age, sex, survey date and location) referents (n=1082, 40% women) free of CVD were recruited from the same database. Glucose intolerance was categorised into impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and diabetes mellitus (DM) based on an OGTT and data on treatment at baseline. The impact of leptin and glucose intolerance on future MI was explored in conditional logistic regression analyses and odds ratios (OR) with 95 % confidence intervals were calculated.
Results: At baseline, both males and females with MI had higher leptin levels (5.0 ng/mL vs. 4.1 ng/mL, p<0.001 and 15.4 ng/mL vs. 14.0 ng/mL, p=0.03) compared to referents. High leptin levels predicted MI in males but not in females, independently of glucose intolerance, 1.9 (1.1-3.0) and 1.0 (0.5-2.0), respectively. After further stratification, leptin predicted ST elevation MI but not non-ST elevation MI in males, 2.9 (1.4-6.4) and 1.4 (0.6-3.6), respectively. After adjustments including leptin, DM and IGT associated with MI, ORs 2.3 (1.3-4.1) and 2.4 (1.3-4.0), respectively. After stratification for sex, DM associated with MI in men and IGT with MI in women, ORs 2.5 (1.4-4.5) and 4.1 (1.4-12.1), respectively.
Conclusion: High leptin and glucose intolerance predict first-ever fatal and non-fatal MI, notably with a clear gender difference.
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Assessment of matrix metalloproteinase-9 as a non-traditional cardiovascular risk marker in prediabetes and newly diagnosed type 2 diabetes

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Background and aims: Matrix metalloproteinase-9 (MMP-9) is a proteolytic enzyme which main substrate is basement membrane collagen and it has been lately recognized as a non-traditional marker of cardiovascular risk. There is growing evidence that MMP-9 plays a key role in extracellular matrix degradation and remodeling and is involved in all stages of the atherosclerotic process. The aim of the present study was to assess MMP-9 levels in prediabetic states - impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), and in newly diagnosed type 2 diabetes (NDD) as well as to evaluate the relationship between MMP-9 and glycaemic control (blood glucose, HbA1c), anthropometric parameters (body mass index, visceral fat area) and common cardiovascular risk factors (hsCRP, serum lipids).
Materials and methods: 249 subjects, distributed into four age-, sex- and BMI-matched groups, were enrolled in the study - 63 subjects with normal glucose tolerance (NGT) (32 males and 31 females, mean age 49.6±14.2 years, mean BMI 30.0±5.0 kg/m^2), 62 subjects with IFG (32 males and 30 females, mean age 49.4±11.1 years, mean BMI 30.6±5.8 kg/m^2), 62 subjects with IGT (31 males and 31 females, mean age 49.0±13.8 years, mean BMI 30.6±6.7 kg/m^2) and 62 subjects with newly diagnosed type 2 diabetes (32 males and 30 females, mean age 48.7±10.6 years, mean BMI 30.4±5.3 kg/m^2). Glucose tolerance was studied during oral glucose tolerance test with evaluation of venous plasma glucose at 0 min and 120 min by a hexokinase method (Roche Diagnostics). hsCRP, lipid profile, MMP-9 and HbA_{1c} were estimated at fasting state. hsCRP was measured turbidimetrically (Roche Diagnostics). Lipid profile (total cholesterol, HDL-cholesterol, triglycerides) was assessed by an enzymatic colorimetric method (Roche Diagnostics). LDL-cholesterol was calculated using Friedewald's formula. MMP-9 was assessed immunoenzymatically (ELISA, CalBiochem) and HbA_{1c} - immunoturbidimetrically (Roche Diagnostics). BMI and visceral fat area were measured with body composition analyzer using eight-point multi-frequency bioelectric impedance analysis (InBody 720, BIOSPACE). Statistical analysis was performed with SPSS 16.
Results: No significant difference in MMP-9 level was found between the groups with NGT and NDD as well as between NGT group and either of the prediabetic groups. No correlation was established between MMP-9 level and glycaemic control parameters. Significant negative correlation was established between MMP-9 and HDL-cholesterol level (r=-0.24, p<0.05). We have found significant correlation between MMP-9 and hsCRP (r=0.482, p=0.04) as well as between MMP-9 and BMI (r=0.391, p<0.01) and visceral fat area (r=0.346, p<0.02).
Conclusion: In early stages of impaired glucose homeostasis - prediabetes and newly diagnosed type 2 diabetes, serum MMP-9 levels do not differ significantly from those in subjects with normal glucose tolerance. In prediabetes and NDD serum MMP-9 correlates mainly with anthropometric (BMI, visceral fat area) and inflammatory (hsCRP) markers and shows weak correlation with parameters of metabolic control (HDL-cholesterol). Probably MMP-9 activity in these states is related to inflammatory changes rather than to metabolic ones.
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High osteoprotegerin serum levels in newly diagnosed type 2 diabetic males with or without known coronary artery disease

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Background and aims: Osteoprotegerin (OPG) is an inhibitor of osteoclastogenesis, but is produced from vasculature too. Recently increased circulating OPG levels were found in diabetics and in patients with coronary artery disease (CAD). Elevated serum OPG appears to be a powerful predictor for cardiovascular mortality. Up to date there are insufficient data for OPG concentrations in newly diagnosed type 2 diabetic patients. The aim of our study was to determine serum OPG in males with newly diagnosed T2DM associated or not with known concomitant CAD and to investigate the relationship between OPG and metabolic components.
Materials and methods: Serum OPG levels were measured in 45 newly diagnosed type 2 diabetic males and 20 age- and BMI-matched normoglycemic male subjects. The newly diagnosed diabetics consisted of 28 diabetics without history of CAD and 17 diabetic patients who underwent percutaneous coronary interventions (PCI) for CAD. Mean intima-media thickness (IMT) of common carotid arteries in diabetics without known CAD was measured by B-mode ultrasonography. All newly diagnosed glucose abnormalities were detected during 2 screening programs among risk groups. Glucose tolerance was defined by performing a standard OGTT. OPG was measured by ELISA (BioMedica).
Results: OPG was significantly higher in newly diagnosed type 2 diabetics compared to controls (4.4 ± 0.2 vs 3.3 ± 0.4 pmol/l; p=0.02) but there was no significant difference between diabetic males with performed PCI or those without known CAD - 4.2± 0.3 vs 4.6 ± 0.3 pmol/l, p=0.41 respectively. In total group of subjects, there was positive correlation of OPG levels with fasting plasma glucose (r=0.37, p=0.008), 120 min post-OGTT glucose (r=0.42, p=0.004) and HbA1c (r=0.50, p=0.0002). Interestingly, in newly diabetic males OPG correlated only with HbA1c (Pr 0.40, p=0.009). Moreover in diabetics without known CAD, OPG correlated significantly with carotid IMT (Pr 0.48, p=0.03) and age (p=0.04). There was no association with fasting insulin, homeostasis model assessment of insulin resistance (HOMA-IR), systolic and diastolic blood pressure, BMI or waist circumference. From lipid parameters OPG showed positive correlation only with HDL-cholesterol (r=0.34, p=0.02).
Conclusion: We found higher serum OPG levels in newly diagnosed type 2 diabetic males independently of presence of known CAD. OPG showed association with glucose parameters, early markers of atherosclerosis and probably they may be involved in the regulation of OPG. We suggest that OPG rises early in the evolution of diabetic disorders but further investigations are needed.
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1244

Serum YKL-40 levels predict the development of vascular complications in patients with type 2 diabetes

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Background and aims: Macrophages in atherosclerotic plaques and vascular smooth muscle cells secrete YKL-40, associated with inflammation, endothelial dysfunction and increased tissue remodeling. Progression of nephropathy with increasing levels of albuminuria has been reported to be intimately linked to an increased risk of cardiovascular (CV) disease. The aim of the present study was to examine the changes in serum YKL-40 levels in patients with type 2 diabetes with increasing levels of albuminuria and macroangiopathy.
Materials and methods: A total of 396 patients with type 2 diabetes were enrolled in this study: 212 males/184 females, age 60±13 years, estimated duration of the disease 12±8 years, body mass index (BMI) 26.0±4.6 kg/m^2,HbA1c 7.4±1.4%. Two hundred fifteen patients had normoalbuminuria, and 122 had persistent microalbuminuria, and 59 had overt proteinuria (macroalbuminuria). Ninety patients showed the past history of CV events (macroangiopathy). The control group consisted of 100 healthy individuals with normal glucose tolerance matched for gender and age. Examination included blood and urine samples for CV risk factors, and markers including lipids, high sensitive C-reactive protein (hsCRP), interleukin (IL)-6 and adiponectin in addition to YKL-40.
Results: Serum YKL-40 levels were positively correlated with age (r=0.333, p<0.0001), systolic blood pressure (r=0.144, p<0.005), serum creatinine (r=0.218, p<0.0001), urinary albumin-to-creatinine ratio (r=0.279, p<0.0001) and IL-6 (r=0.243, p<0.0001). However no significant correlation was found between YKL-40 and parameters such as BMI, HbA1c and hsCRP. Serum levels of adiponectin were not related to levels of YKL-40 in patients with normoalbuminuria (r=0.005.p=0.9405) but both parameters interestingly showed significant correlation in patients with microalbuminuria (r=0.266, p<0.005). YKL-40 levels had no gender differences, and were significantly elevated in patients with type 2 diabetes compared with control subjects (135.8±94.7 vs. 80.7±58.3 ng/ml; p<0.0001). YKL-40 levels were evidently increasing with advancing stages of nephropathy (normoalbuminuria 105.2±74.0, microalbuminuria 156.5±98.0 and macroalbuminuria 205.3±107.8 ng/ml; p<0.005 for all comparisons). Patients with the past CV events showed significantly higher YKL-40 levels than other patients (165.4±99.4 vs. 126.3±91.5 ng/ml; p<0.001).
Conclusion: YKL-40 levels are significantly elevated in patients with type 2 diabetes with advancing stages of nephropathy, and also in patients with macroangiopathy. These findings suggest that YKL-40 has the usefulness as a novel marker predicting the progressing vascular complications in patients with type 2 diabetes.

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Insulin resistance: a risk marker in coronary population without known diabetes

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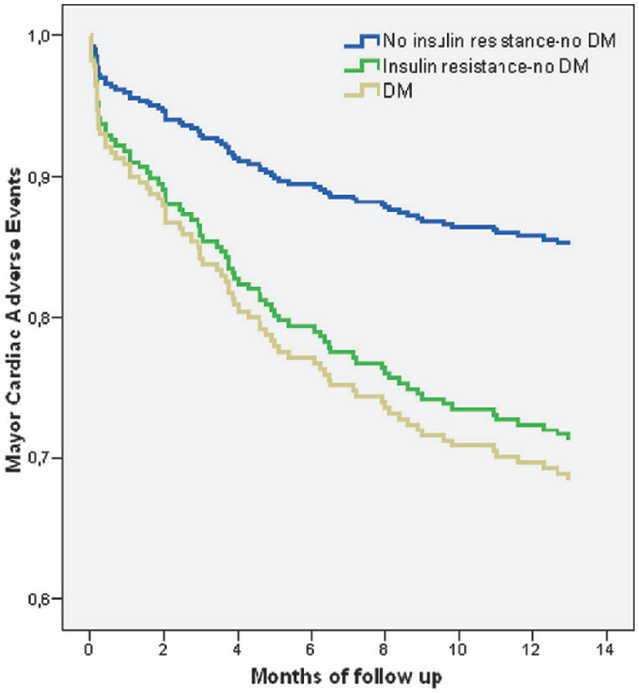
Background and aims: The impact of clinically established diabetes in the prognosis of the patients with coronary disease is well known. However, contradictory data exist about the prognosis if these patients present unknown diabetes, newly detected diabetes or prediabetes. As the phisiopathologic substrate is the insulin resistance the purpose is to validate its role as prognosis factor in our series.
Materials and methods: We studied a cohort of 472 patients with coronary disease [(who underwent percutaneous coronary intervention (PCI)]. In those 338 patients without known diabetes an analysis including fasting plasma glucose, oral glucose tolerance test, glycated hemoglobin, insulinemia, and renal and lipid profiles was performed 15 days after discharge. The real glycometabolic profile of the population was addressed following the OMS criteria of 1999. The HOMA was calculated with Matthews´formula. Insulin resistance was defined as HOMA>3 (Sekiguchi et al). A composite end-point of major cardiac events (MACE) that included death, non-fatal AMI, new PCI and stroke was registered after a 12-month follow-up. Kaplan-Meier and multivariable analysis were performed to determine the predictors of MACE.
Results: Age: 66.5 (56-74), males 80.1%, active smokers 28.4%, hypertension 49.7%, obesity 35.5%, peripheral or cerebrovascular disease 15.4%. The real distribution of the cohort after the glycometabolic study was: known diabetes 28.8%, newly detected diabetes 16.2%, prediabetes 25.5% and normoglycemic 29.5%. Forty patients were classified as resistance to insulin and 298, non insulin resistance. In the multivariable analysis the predictors of MACE were the presence of known diabetes (30.6%) and insulin resistance (27.5%). The MACE in non insulin resistance, normoglycemics, prediabetes and newly detected diabetes were 11%, 10.7%, 10.7% and 12.9%, respectively. (See Kaplan-Meier curve, DM is known diabetes).
Conclusion: In our cohort of patients with coronary disease who underwent PCI the presence of known diabetes and the insulin resistance were predictors of MACE at 12 months. Prediabetes and newly detected diabetes did not increase the risk of MACE in comparison with the normoglycemics.



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1246

Abdominal obesity, hypertension and cardiovascular risk in diabetic patients: Poland compared to North-West Europe Region - insights from IDEA sub-study

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Background and aims: The cluster of anthropometric and metabolic disorders define the risk of cardiovascular disease (CVD). The IDEA (International Day for Evaluation of Abdominal Obesity) study was an international cross sectional study including 168159 primary care patients in 62 countries. The aims of the present report were: (i) to compare the prevalence of abdominal obesity (AO), hypertension (HT) and cardiovascular disease (CVD) in Poland-PL and North-West Europe Region -NW (Austria, Belgium, Denmark, Finland, France, Germany, Ireland, Netherlands, Norway, Sweden, Switzerland) in diabetic (DM) sub-population of IDEA study; (ii) to assess the impact of DM and AO, HT on CVD in Polish population.
Materials and methods: In Poland, 200 randomly selected general practitioners included 5371 consecutive patients, aged 18 to 80 years, 2024 men and 3347 women. Waist circumference (WC), BMI, the presence of DM, HT and CVD (defined as coronary heart disease, stroke, or revascularization) were recorded. AO was diagnosed according to the NCEP criteria (WC >102 cm for male (M) and >88 cm for female (F).
Results: The prevalence of DM in PL vs. NW was 12.7% vs. 12.9% in M and 10.8% vs. 8.8% in F (p<0.001), respectively. The prevalence (%) of abnormalities in risk factors in diabetic sub-population is shown in the table. . In multiple logistic model, age (OR=1.075, 95% C.I. 1.07-1.08, P<0.001), AO (OR=1.41, 95% C.I. 1.15-1.73, P<0.001), male gender (OR= 1.60, 95% C.I. 1.37 - 1.85, P<0.001), DM (OR= 1.70, 95% C.I. 1.4 - 2.06, P<0.001) and HT (OR= 3.15, 95% C.I. 2.7 - 3.68, P<0.001) were independent predictors for CVD in Poland. The impact of AO, HT and DM on CVD were independent of gender (P>0.1 for interaction). DM added to combination of AO and HT increased the aged-adjusted probability of CVD (95% C.I) from 41% to 54% in M (p<0.001) and from 30% to 42% in F (p<0.001), respectively.
Conclusion: The population profile of cardiovascular risk factors in diabetic patients is worse in Poland when compared with North and Western Europe. This difference was more striking for women. These results contribute to increased burden of CVD, and require novel intensive preventive strategies in Poland.

Table

	Male		Female	
	PL n=257)	NW (n=1646)	PL (n=364)	NW (n=1473)
BMI>30 kg/m^2	56**	43	59**	51
AO	65	59	85*	80
HT	76	72	77	73
CVD	62**	41	55**	35
	*p<0.05; ** p<0.001 vs. NW			

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Gender differences in the progression of atherosclerosis of carotid and coronary arteries using MDCT and carotid ultrasonography

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Background and aims: Cardiovascular disease (CVD) is a leading causes in death with type 2 diabetes and it is important to prevent onset of CVD. One of the clinical intervention and screening of CVD is multiple detector-row computed tomography (MDCT) to detect the progression of atherosclerosis. Ultrasonic evaluation of early carotid is also a useful tool for the prediction of occurrence of ischemic cerebrovascular events. We are routinely using these examinations for early detection of atherosclerotic evaluation and intervention with medical treatment. Here, we tried to clarify the significance of carotid ultrasonography and MDCT in gender in order to screen the atherosclerotic changes.
Materials and methods: A total of 115 patients (men: 74, women: 41) with type 2 diabetes attending Yachiyo Medical Center,Tokyo Women's University were studied. Mean patients ages were 64.4 years old for men and 66.8 for women. Mean body mass index (BMI) was 24.2±3.4 kg/m^2. Ultrasound examination for carotid intima-media thickness (IMT), max IMT, plaque character, number (plaques score) and echolucency were used. The plague score was calculated by summing the maximum of intima-media complex (plaque thickness) measured in millimeters on the near and far walls at each of four divisions of both sides of the carotid arteries. Quantification of coronary artery calcium was examined according to Agaston scores.
Results: Plaque score in men was 8.03±0.70 and 6.37±0.60 in women and there was no significant difference. Calcium score in coronary arteries in men was 630.4±156.5, and 157.8±34.6 in women and there was a significant difference between them (P<0.05). There was a tendency to increase in a ratio of calcium score and plaque scores in men compared with in women, but there was not significant difference (P=0.06). Not calcium score but plaque score was positively significantly correlated with the duration of diabetes (p<0.05). Calcium scores were positively correlated with numbers of echorich plaques in both men and women (P<0.01) but not with echolucent plaques. Interestingly, significant correlation between calcium score and plaque score was not observed in total subjects (r=0.12, P=0.21) and there was no correlation in men (r=0.07, P=0.52), but there was strong correlation in women (r=0.389, P<0.01).
Conclusion: Carotid ultrasonography is a useful non-invasive screening for atherosclerotic evaluation and prediction for coronary and cerebrovascular events, but there is a significant correlation between plaque score and calcium score in MDCT in women, but not in men. Carotid ultrasonography is predictive for coronary atherosclerosis in women suggesting that the investigation of active coronary atherosclerosis examination is necessary regardless of severity of carotid ultrasonograhy results in men.

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Screening strategy for asymptomatic coronary heart disease in Japanese patients with diabetes mellitus

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Background and aims: Although type 2 diabetes has been indicated to be equivalent risk to myocardial infarction in Finnish study, Japanese subjects with type 2 diabetes has been indicated to be less frequently associated with coronary heart disease (CHD) than Caucasians. In the present study we investigated screening methods of asymptomatic CHD with type 2 diabetes.
Materials and methods: All patients with type 2 diabetes of our outpatient department (OPD) (n=558) were checked with electrocardiography (ECG) at rest, and all of inpatients with type 2 diabetes (IN) (n= 573) were checked with both ECG at rest and Treadmill tolerance test (TTT). Those with previous history of CHD or contraindication for TTT were excluded. The OPD patients with abnormal ECG findings were investigated with TTT and/or thalium 201 cardiac scanning (TCS). Thereafter the subjects (both IN and OUT) with abnormal TTT and/or TCS findings were examined with coronary angiography (CAG) in order to make a final diagnosis of CHD.
Results: Among 558 OPD patients, 134 subjects had abnormal ECG at rest, and 52 of them received TTT and/or TCS. A total of 4 subjects received CAG and all of them were finally indicated to have CHD. Among 573 IN patients, a total of 70 had positive TTT, and 61 patients received TCS. Among 61 patients, a total of 22 (36.1%, ie 3.8 % of total) had positive TCS. There were no significant differences of basal clinical parameters between the TCS-positive group and TCS-negative group, except for female dominance in negative group.Thirty-seven (52.9%) of TTT-positive patients and 10 (45.5%) of TCS-positive patients had one or more of the following; abnormal ECG at rest, history of other macroangiopathy, or more than two cardiovascular risk factors. Among TCS-positive patients, a total of 14 subjects received CAG, and eight of them (ie 57.1%) were finally indicated to have CHD; five cases received percutanous coronary intervention (PCI), two cases received coronary bypass surgery (CABG), and the remaining one case was followed with medication.
Conclusion: These results suggest that a risk factor-guided screening approach for asymptomatic CHD may not be sufficiently adequate, at least, in Japanese patients with type 2 diabetes.

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Diabetic retinopathy is a risk factor for cardiovascular disease in Japanese patients with type 2 diabetes. The Otowa Hospital Diabetes Observational Study 2 (OHDOS 2)

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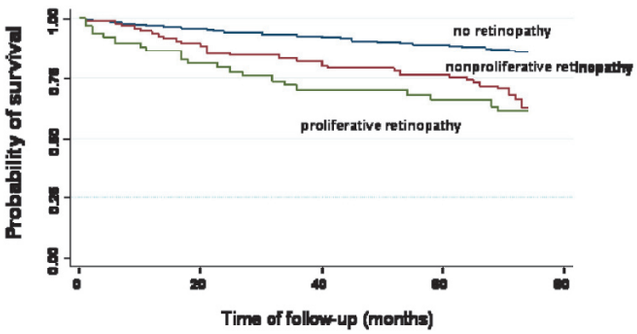
Background and aims: Type 2 diabetes is a potent risk factor for cardiovascular disease (CVD). Recently microvascular complications of diabetes are increasingly recognized as independent risk factors for CVD. For example, there is increasing evidence that nephropathy has strongly and independently been associated with the development of CVD. Diabetic retinopathy is also increasingly recognized as an independent CVD risk factor, but there is less evidence than nephropathy. Therefore, the aims of our study were to further explore the relationship of retinopathy with CVD in Japanese patients with type 2 diabetes.
Materials and methods: Overall 568 consecutive outpatients with type 2 diabetes who came to Rakuwakai Otowa Hospital in Kyoto, which is a teaching hospital with 588 beds, in 2003 were studied retrospectively for 6 years or until they experienced a cardiovascular event or died. CVD during follow-up was defined as myocardial infarction, angina, silent myocardial ischemia and stroke. Retinal findings were classified into three categories according to the status of the more impaired eye: no retinopathy, nonproliferative retinopathy, and proliferative retinopathy. Statistical analysis was performed by Cox proportional hazard model to estimate hazard ratios of retinopathy.
Results: The mean age of 568 patients was 63±12 years. Approximately 38% of patients were women (350 men and 218 women). Median duration of diabetes were 10.0±9.7 years, and 21% of patients were taking aspirin. The mean glycated hemoglobin (HbA1c) was 7.8%. One hundred four patients had nonproliferative retinopathy (18.3%) and 69 patients had proliferative retinopathy (12.1%). After 6 years follow-up, 102 patients had incident CVD (18.0%). Figure 1 shows Kaplan-Meier curves for the cumulative incidences of CVD by the grade of retinopathy. After 6 years follow-up, both nonproliferative retinopathy (Cox model hazard ratio [HR], 2.58; 95% confidence interval [CI] 1.58 to 4.20; p<0.001) and proliferative retinopathy (HR 3.59; 95% CI 2.00 to 6.44; p<0.001) had increased CVD risks. These associations were independent of sex, age, duration of diabetes, history of smoking, LDL cholesterol, HDL cholesterol, chronic kidney disease (CKD), hypertension, and history of CVD. In the group of patients with history of CVD, HRs of incident CVD were 2.29 (95% CI 1.07 to 4.89; p=0.032) and 3.26 (95% CI 1.39 to 7.64; p=0.007) in patients with nonproliferative and proliferative retinopathy. In the group of patients without history of CVD, both nonproliferative retinopathy (HR 2.98; 95% CI 1.54 to 5.75; p=0.001) and proliferative retinopathy (HR 4.00; 95% CI 1.67 to 9.61; p=0.002) had strongly increased CVD risks.
Conclusion: This study shows that Japanese type 2 diabetes patients with nonproliferative or proliferative retinopathy had an increased risk for incident CVD.

Figure 1. Kaplan-Meier survival estimates, by retinopathy



PS 124 Cardiac complications

1250

Targeting intensive glycaemic control versus conventional glycaemic control in type 2 diabetes mellitus - a meta-analysis of 29,000 patients

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Background and aims: Patients with type 2 diabetes mellitus (T2D) have increased mortality primarily due to increased risk of cardiovascular disease (CVD). Epidemiological studies suggested an association between elevated blood glucose and the development of both micro- and macrovascular complications. However, recent randomised clinical trials (RCTs) have questioned the benefit of intensive glucose control. The aim of this systematic review was to assess the effects of targeting intensive versus conventional glycaemic control in patients with T2D.
Materials and methods: RCTs that prespecified different targets of blood glucose control in the intervention arms were identified through searches of The Cochrane Library, MEDLINE, EMBASE, Science Citation Index Expanded, LILACS and CINAHL. We contacted relevant companies, experts and conference proceedings from major diabetes congresses. RCTs in adults with T2D irrespective of language and publication status were included. Two authors independently extracted data. If needed additional information was obtained from authors.
Results: Nineteen RCTs were included, randomising 29,977 patients with T2D (16,085 to intensive control, 13,860 to conventional control). The mean duration of the intervention varied from 3 days to 12.5 years. The relative risk (RR) for the primary outcomes of all-cause mortality (fixed RR 1.00, 95% CI 0.93 to 1.08) or CVD mortality (fixed RR 1.05, 95% CI 0.95 to 1.17) was not significant. Meta-regression for all-cause mortality showed no significant influence of disease duration, HbA1c or fasting blood glucose at baseline. However, the risk of all-cause mortality was negatively correlated to duration of the intervention (P= 0.08). The risk of CVD mortality was not influenced by the variables explored by meta-regression. Intensive glycaemic control significantly reduced the risk of non-fatal myocardial infarction (fixed RR 0.86, 95% CI 0.78 to 0.96; P=0.006) and amputation of lower extremity (fixed RR 0.64, 95% CI 0.44 to 0.95; P=0.03). The RRs of non-fatal stroke, cardial revascularization and peripheral revascularization were not significant. Intensive glycaemic control reduced the risk of all microvascular complications (fixed RR 0.85, 95% CI 0.78 to 0.93; P=0.0003), including nephropathy (fixed RR 0.80, 95% CI 0.70 to 0.91; P=0.0007) and retinal photocoagulation (fixed RR 0.79, 95% CI 0.69 to 0.91; P=0.002). Non-hypoglycaemic serious adverse events were more common when targeting intensive glycaemic control (fixed RR 1.06, 95% CI 1.02 to 1.11; P=0.003). The risk of severe hypoglycaemia was increased when targeting intensive glycaemic control (fixed RR 2.71, 95% CI 2.42 to 3.02; P<0.00001). The cost-effectiveness of intensive glycaemic control was neutral. It was not possible to meta-analyse quality of life.
Conclusion: There is insufficient evidence to determine whether targeting intensive glycaemic control versus conventional glycaemic control reduces all-cause mortality and CVD mortality. Meta-regression showed negative correlation between duration of the intervention and all-cause mortality. Intensive glycaemic control reduces key clinical, including all microvascular outcomes. However, conventional glycaemic control reduces the risk of severe hypoglycaemia and other serious adverse events.
Supported by: CIMT Group

1251

Differences in the short-term medium-term and long-term outcomes between newly diagnosed diabetes patients known diabetic and prediabetic patients after an acute coronary syndrome

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Background and aims: Diabetes is a major contributor to cardiovascular diseases, as well as an independent predictor for adverse outcomes in patients after an Acute Coronary Syndrome (ACS). The impact of different categories of glucose metabolism on patient outcome after discharge varies according to elapsed time after ACS. The aim of this study is to determine the correlation of these categories with the incidence of short-term and long-term complications after an ACS.
Materials and methods: 520 patients mean aged 66.14±11.94 years that were admitted to the coronary care unit and discharged were included in this longitudinal, prospective, observational, study. The study's end- points were: death (of cardiovascular cause), myocardial infarction, cardiac failure (clinical and echocardiographic determination) and unstable angina after hospitalization. Short-term was defined at 30 days after discharge, medium-term at 6 months, and long-term at 12 months. Non-diabetic patients went through an Oral Glucose Tolerance Test one month after discharge and IGTs were categorized. Adjusted and unadjusted logistic regression analyses were carried out in order to find the correlation between the glycemic status of the patients and the incidence of complications during the first 30 days and one year after their discharge.
Results: Out of the study's 520 patients, diabetes was previously diagnosed (Group A) in 152 (29.2%) patients, newly diagnosed (Group B) in 57 (10.9%) patients, and IGT (Group C) was observed in 110 (21.1%) patients while 201 (38.8%) were patients with normal glucose regulation (Group D). Regarding the patient outcome after the first 30 days following ACS, group B had the worst outcome (HR:2.15, 95%CI: 1.109-4.156, p=0.001), followed by groups A (HR:1.87, 95%CI: 1.228-5.231, p=0.003) and C (HR:1.22, 95%CI: 0.976-2.985, p=0.112) using group D as a reference group after adjustment for age, gender, smoking, waist circumference, HDL, triglycerides, total cholesterol, metabolic syndrome (NCEP-ATP III criteria) and hypertension. According to patients' outcome six months after ACS, group A shows the worst outcome (HR: 2.05, 95%CI:1.211-5.781, p=0.001) followed by group B (HR:1.92, 95%CI:1.149-4.483, p=0.001) and group C(HR:1.23, 95%CI:0.985-3.244, p=0.109) using group D as reference category. There was no difference between group A and B (p=0.244). Concerning patient outcome during the first 12 months after ACS, group A showed the worst outcome (HR:2.66, 95%CI:1.234-5.135,p=0.001) followed by groups B(HR:1.84, 95%CI:1.129-4.328,p=0.022) and C(HR:1.25, 95%CI: 1.115-3.289,p=0.046) using group D as reference group after adjustment to the before mentioned factors.
Conclusion: Newly diagnosed diabetic patients with ACS show a worse short-term outcome compared to known diabetic patients due to the fact that those patients have diabetes that was neither appropriately recognized nor treated before hospitalization. Patients with known diabetes mellitus have a worse long-term outcome after ACS compared with newly diagnosed and IGT patients, while there are no differences between known and newly diagnosed diabetes patients in medium-term outcome.

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Ethnic differences in the prevalence of cardiovascular disease and its risk factors in subjects with and without diabetes in Oslo, Norway

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Background and aims: The population in Oslo is multiethnic due in large part to immigration from Asia and Africa in the last decades. In these ethnic minority groups, we have previously reported a high prevalence of diabetes, diabetes was diagnosed at a younger age and the glycaemic control was poorer compared to Norwegians. The aim of the present study was to assess the prevalence of self-reported diabetes and cardiovascular disease (CVD) and its risk factors related to diabetes status in five minority groups compared with the Norwegians.
Materials and methods: Data from three population-based health surveys conducted in Oslo between 2000 and 2002 were merged. Of 54473 invited individuals 24749 (45.4%) participated. Our study was restricted to participants born in Norway, Turkey, Iran, Sri-Lanka, Pakistan and Vietnam between 1940 and 1971 (N=18417). Data about self-reported diabetes, CVD (coronary heart disease and/or stroke), physical inactivity, body mass index (BMI), blood pressure (BP), glucose and lipids for subjects with and without diabetes were analyzed. We used country of birth as proxy for ethnicity as the minorities in this study are mainly 1. Generation immigrants. Chi-square testes, multiple regression and logistic regression were used.
Results: Of the 17854 study subjects with known diabetes status, 562 reported diabetes. Table 1 gives the age, ethnic composition, disease prevalence and risk factors of the study population. The prevalence of self-reported diabetes varied from 1.9 % (Norwegians) to 13.3% (Pakistanis), p<0.001, and the eth-

nic minority groups reported more CVD (4.1 to 7.6%) compared to Norwegians (2.9%, p<0.001), despite being younger. Ethnic differences were found for most risk factors irrespective of diabetes status. For subjects not reporting diabetes, the OR for CVD adjusted for age and gender was higher in all the ethnic minority groups (2.6 to 4.1, p<0.001) compared with Norwegians, whereas for subjects with diabetes OR for CVD was only significantly higher compared to Norwegians for the Vietnamese 4.0 (p=0.01).
Conclusion: All ethnic minority groups reported higher prevalence of diabetes and CVD than Norwegians. The excess risk of CVD in ethnic minorities was more profound in subjects not reporting diabetes than in those with diabetes, indicating the need to improve primary prevention of CVD in these groups.

Table 1. Crude prevalence of self-reported diabetes, CVD and risk factor levels by ethnicity

	Norway (n=13967)	Turkey (n=548)	Iran (n=695)	Sri-Lanka (n=1127)	Pakistan (n=859)	Vietnam (n=658)	P ANOVA
Age,years Mean (95% CI)	45.2 (45.0-45.4)	41.5 (40.9-42.2)	41.6 (41.1-42.1)	39.6 (39.2-39.9)	43.3 (42.7-43.9)	43.2 (42.6-43.8)	<0.001
Men (%)	44.5	55.3	59.9	60.0	54.2	45.7	<0.001
Self-reported diabetes (%)	1.9	6.0	2.4	8.8	13.3	5.8	<0.001
Self-reported CVD (%)	2.9	6.4	6.1	4.1	7.6	7.2	<0.001
Systolic blood pressure Mean (mmHg)	136.8	129.7	125.9	126.5	132.2	123.3	<0.001
Diabetes, yes							
Diabetes, no	128.3	122.1	120.3	121.7	123.6	120.3	<0.001
Cholesterol/ hdl-cholesterolratio Mean	4.5	4.9	4.7	4.9	5.1	4.4	0.005
Diabetes, yes							
Diabetes, no	4.0	4.7	4.5	4.9	4.8	4.1	<0.001
BMI > 25kg/m2 (%)	77.1	93.9	82.4	62.9	92.0	55.3	<0.001
Diabetes, yes							
Diabetes, no	49.7	78.1	62.2	57.1	76.4	26.9	<0.001
Physical inactivity (%)	26.8	71.4	71.4	48.1	59.1	43.8	<0.001
Diabetes, yes							
Diabetes, no	21.1	56.3	46.3	53.6	56.5	58.1	<0.001
Current smoker (%)	25.3	53.1	23.5	11.8	12.1	16.2	<0.001
Diabetes, yes							
Diabetes, no	28.9	39.6	35.1	11.9	21.1	18.4	<0.001

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The effect of different glucose values during hospitalisation on one-year outcome after an acute coronary syndrome

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Background and aims: Elevated glucose at the time of hospital admission is associated with increased mortality rates among patients hospitalized with Acute Coronary Syndrome (ACS). Admission glucose represents only a single measurement in time while in-hospital hyperglycemia during the first days after admission or during the entire ACS hospitalization period is obtained by the use of multiple glucose values. The aim of this study is to evaluate the impact of different glucose values (admission, fasting, postprandial, mean hospitalization glucose and HbA1c) for these patients' first-year outcome.
Materials and methods: 520 patients were admitted to the coronary care unit and discharged February 2006 - October 2007 were included in this longitudinal, prospective, observational, study. First-year end points were: death (of cardiovascular cause), myocardial infarction, cardiac failure (clinical and echocardiographic determination) and unstable angina after hospitalization. Non-diabetic patients went through an OGTT one month after discharge and IGTs were categorized. To evaluate the impact of different glucose measurements on first-year complications after the ACS incidence, separate logistic regression models were performed for each measurement. The accuracy of these logistic regression models in predicting complications for the predetermined time period was assessed using the Receiver- Operating Characteristic (ROC) curves, and their respective areas under the curve (AUC).
Results: Diabetes was previously diagnosed (Group A) in 152 (29.2%) patients, newly diagnosed (Group B) in 57 (10.9%) patients, an IGT (Group C) was observed in 110 (21.1%) patients while 201 (38.8%) were patients with normal glucose regulation (Group D). The incidence of one-year complications was 24.3%, 21.1%, 13.6% and 11.9% in groups A, B, C and D respectively (p=0.014). AUC of ROC curves for the probabilities of the logistic regression models (adjusted for age, gender, smoking, waist circumference, HDL, triglycerides, total cholesterol, metabolic syndrome (NCEP-ATP III) and hypertension) for the admission glucose was 0.697 (p=0.001) 0.627 (p=0.010) 0.601 (p=0.022) and 0.578 (p=0.049) for A, B, C and D Group respectively. AUC for the fasting glucose was 0.632 (p=0.021), 0.581 (p=0.033) for groups A, B respectively. Statistically significant AUC for the postpandial glucose was just for Group A [0.611, (p=0.033)] while significant AUC for the mean value of the glucose during hospitalization was 0.601 (p=0.026) 0.578 (p=0.036) and 0.557 (p=0.048) for Groups A, B and C respectively. AUC for the HbA1c was 0.592 (p=0.033) and 0.522 (p=0.043) for groups A and B respectively, showing no statistical significance as to the outcome of the other two groups.
Conclusion: Admission glucose in ACS incidents shows the most significant correlation with first-year end-points even in normoglycemic patients among different glucose values during hospitalization. The postprandial glucose is related with end-points only in known diabetics while the fasting glucose shows a significant correlation only in diabetic patients. Prolonged hyperglycemia expressed by mean glucose values during hospitalization affects newly diagnosed, known diabetic patients and IGT patients.

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Evidence of diastolic dysfunction in NAFLD: A study using tissue Doppler echocardiography

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Background and aims: Non alcoholic fatty liver disease (NAFLD) is considered the hepatic manifestation of metabolic syndrome and such patients have increased risk of cardiovascular events (myocardial infarction, stroke, coronary revascularisation and cardiovascular death). Recent data suggests that patients with NAFLD had a hazard ratio of 2 for developing a cardiovascular endpoint in 6.5 years. Abnormalities in diastolic function often presage cardiac disease. Diastolic dysfunction may progress to symptomatic heart failure without systolic impairment. The aim of this study is to compare diastolic dysfunction in patients with NAFLD compared with age matched controls using tissue Doppler echocardiography, a method which can measure differential velocities within a region of interest.
Materials and methods: 15 patients with NAFLD and 15 normal controls were recruited. The diagnosis of NAFLD was as per local guidelines. Subjects underwent transthoracic echocardiography using a GE Vivid Q machine (2.5MHz phased array transducer). Colour tissue Doppler loops (3 cardiac cycles) in each of the apical 4-chamber, 2-chamber and long axis imaging planes were acquired triggered to the ECG and saved digitally for subsequent off line analysis by a single experienced operator data who produced the strain and strain rate curves using Echopac V9.01 (GE, Horten, Norway).
Results: The control and NAFLD groups were matched for age, BMI and systolic blood pressure: 50.8±8.6 vs 48.4±13.2 years, 28.1±5.0 vs 28.1±4.9kg/m^2, 126.4±12.8 vs 128.6±8.8mmHg. Nor was there a significant difference in ejection fraction 57.1±12.3 vs 62.4±8.9% or left ventricular mass index 74.1±14.2 vs 79.8±15.1.E/Ea ratio is significantly elevated in patients with NAFLD (table 1). E/Ea ratio is a marker of LA pressure which acts as a surrogate for diastolic dysfunction. Patients with NAFLD showed significant reductions in both diastolic velocities and peak early diastolic strain rate compared with normal controls, supporting the presence of diastolic dysfunction. There is also a non significant trend suggesting patients with NAFLD have reduced systolic velocity and strain.
Conclusion: These results suggest diastolic dysfunction in patients with NAFLD compared with controls. Diastolic dysfunction, left untreated, may

progress to heart failure which may explain the excess cardiovascular events in this group.

Table 1: Tissue doppler results for the left ventricle

	Controls (n=15)		NAFLD (n=15)		p
	mean	sd	mean	sd	
Mean early left ventricular inflow velocity/mean early diastolic myocardial velocity at the septum (E/Ea)	10.5	2.5	13.0	3.1	0.02
Peak systolic myocardial velocity (cm/s)	6.5	.9	5.8	1.2	0.08
Peak early diastolic myocardial velocity (cm/s)	7.9	2.1	6.3	1.8	0.04
Peak late diastolic myocardial velocity (cm/s)	10.1	1.3	7.2	2.9	0.002
Peak systolic strain (%)	22.9	4.4	20.7	3.3	0.14
Peak systolic strain rate (1/s)	1.4	0.4	1.4	0.3	ns
Peak early diastolic strain rate (1/s)	2.4	0.5	1.8	0.4	0.002
Peak late diastolic strain rate (1/s)	2.0	0.5	1.6	0.4	0.06

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Alterations in diastolic function and lipid metabolism occur with the onset of overt hyperglycaemia in women with prior gestational diabetes

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Background and aims: Heart failure is still the main cause of death in patients with type 2 diabetes and especially women with diabetes have an increased cardiovascular risk compared to their male counterparts. Since cardiac complications are often present at the time of diagnosis of diabetes mellitus type 2 the question appeared if alterations in cardiac function are already present in prediabetic stages or if the diagnosis of diabetes is often delayed. Since women with prior gestational diabetes (pGDM) display a young, female population at increased risk for developing type 2 diabetes, the aim of this study was to investigate cardiac function via magnetic resonance (MR) imaging in women with pGDM with normal glucose tolerance, impaired glucose tolerance and overt type 2 diabetes as well as in women with no history of gestational diabetes and normal glucose tolerance (CON).
Materials and methods: ^{1}H magnetic resonance imaging of the myocardium, an oral glucose tolerance test for the assessment of glucose tolerance and blood sampling for the measurement of HbA1C and lipid profile were performed in 8 pGDM with normal glucose tolerance (NGT), 6 pGDM with impaired glucose tolerance (IGT), 12 pGDM with type 2 diabetes (DM) and 10 women with no history of gestational diabetes and normal glucose tolerance during pregnancy (CON), who served as controls. The median follow-up period since pregnancy was 10 years.
Results: DM showed a significant reduction of the E/A-ratio compared to IGT (1.09 vs 1.9; p=0.002) as well as a decreased stroke volume compared to all other groups (60.1±14.5 vs 79.47±14.6 ml;p=0.008) and significantly higher triglycerides (p=0.03) and lower HDL-values compared to NGT and CON (CON:64.8±9.2, NGT:66.4±17.6, IGT:53.5±12.8, DM:46.8±12.0; p=0.0047). E/A ratio and stroke volume were inversely correlated with systolic blood pressure (R=-0.6; p=0.002), triglycerides (R=-0.5; p=0.003) and HbA1C (R=-0.5; p=0.001) and positively correlated with HDL (R=0.4; p=0.03). DM were older compared to NGT and IGT, but comparable to CON. There was no difference in BMI between the groups.
Conclusion: According to our results women with overt diabetes are characterized by alterations of diastolic cardiac funtion and lipid metabolism. The latter seems to develop at the prediabetic stage. Hence, early detection of overt hyperglycemia and dyslipidemia in women at high risk should be the primary aim in the prevention of cardiac complications in diabetes mellitus type 2.

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Effects of hyperglycaemia and hyperinsulinaemia on cardiac function and lipid metabolism: A magnetic resonance spectroscopy and imaging study

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Background and aims: Diabetic cardiomyopathy is a disease specific entity which is present in patients with diabetes even in the absence of coronary artery disease and arterial hypertension. The pathophysiology of this disease is still unknown. However, recent evidence suggests that increased myocardial lipid accumulation (lipotoxicity) likely contributes to its development. Although our recent investigations confirmed increased myocardial lipid content in patients with type 2 diabetes, we could not find any evidence for cardiac steatosis in prediabetic subjects. Therefore we hypothesized that myocardial lipid accumulation might be linked to overt hyperglycemia .Therefore the aim of this study was to investigate the impact of hyperglycemia and hyperinsulinemia during a 6-h clamp on cardiac function and intramyocellular lipids in vivo by non-invasive magnetic resonance (MR) imaging and spectroscopy.
Materials and methods: Hyperglycemic (~ 200 mg/dl, 6h) clamps were performed in 8 healthy subjects (5 males, 3 females; BMI: 22,8±2,9 kg/m^2; age: 29,7±7,2 a). ^{1}H magnetic resonance imaging and breath movement navigated and ECG triggered localized ^{1}H single voxel MR spectroscopy (TE= 30ms) were used to measure left ventricular dynamic parameters and myocardial lipid accumulation in cardiac septum at baseline and after 6 hours of hyperglycemia.
Results: During hypertglycemia myocardial lipid content increased by 27,7% (p=0.04) and this increase in myocardial lipids was inversely correlated with changes in stroke volume (R=-0.82; p=0.02). Furthermore, a small increase in ejection fraction (+6.2%; p=0.007) was observed.
Conclusion: Our preliminary results suggest that hyperglycemia induces cardiac lipid accumulation in healthy subjects and might lead to impaired diastolic myocardial function.

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Are revascularisation procedures in asymptomatic diabetic patients with myocardial ischaemia beneficial? A retrospective study

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Background and aims: Screening diabetic patients for silent myocardial ischemia (SMI) is controversial but some studies have suggested that revascularization may improve the prognosis of the patients with silent coronary artery disease (CAD). The aim was to determine in a retrospective study if coronary revascularization may improve the prognosis of patients with silent CAD.
Material and methods: Between 1992 and 2008, a total of 787 asymptomatic diabetic patients with a normal ECG at rest but with at least another risk factor were screened for SMI by performing a stress myocardial scintigraphy. In the present study we included the 263 patients with SMI (169 men, diabetes duration 14±8 years, nephropathy 45%, hypertension 72%, dyslipidemia 67%, smoking 29%, other artery disease 14%, family history of premature CAD 11%). Coronary angiography was performed in all of them and 93 had silent CAD (1-/2-/3-vessel disease: 49/19/17 patients; and 8 patients with an unknown number of vessel disease). The incidence of a first cardiac event was compared in the patients with SMI but no CAD (group SMI-no CAD, n=171) and in those with SMI and CAD with initial coronary revascularization (group CAD-revascularization: 29 percutaneous coronary intervention (PCI) and 7 coronary artery by pass (CABG)) or without initial revascularization (group CAD-medical, n=56).
Results: The proportion of men was higher in the CAD-revascularization group than in the CAD-medical group (83 vs 54%, p<0.05) with no other clinical or biological significant difference at baseline across both groups. After a mean follow-up of 5.5±4.2 years, 36 cardiac events occurred: 8 cardiac deaths, 23 acute coronary syndromes, 3 secondary coronary revasculariza-

tions, 1 cardiac failure and 1 ventricular fibrillation. The incidence of events differed significantly between the three groups (Kaplan Meier analysis: log rank 23.0, p<0.0001). It was the lowest in the SMI-no CAD group, the highest in the CAD-medical group and intermediate in the CAD-revascularization group. In the patients with 3-vessel disease, the incidence of cardiac events was lower in those who were revascularized by CABG than in those who were medically treated (log rank 6.5, p<0.05).
Conclusion: The cardiac prognosis is poor in the diabetic patients with silent CAD. The data suggest that CABG may improve the prognosis of those with 3-vessel disease.

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The role of soluble ST2 in diabetic patients with preserved left ventricular systolic function and the parameters that influence its value
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Background and aims: Soluble ST2 is a member of the IL-1R receptor family and it has been used in several studies as a sensitive biomarker of cardiac failure. This biomarker has never been studied before in diabetic population, compared to healthy controls. The aim of this study was to find any possible differences of the value of sST2 among diabetic and non diabetic subjects with preserved LV systolic function. The second aim was the revelation of any other biological parameters of diabetic patients that impact on the value of sST2.
Materials and methods: We recruited 106 subjects, 36 healthy and 70 diabetic volunteers that underwent extensive ultrasonographic check of their cardiac function, using the latest revised guidelines of ACC/AHA. All subjects that had an ejection fraction < 50% were excluded. Exclusion criteria were also subjects with history of active malignancy and/or chemotherapy, chronic use of corticosteroids or thiazolinediones and history of autoimmune diseases. We measured all the classical biomarkers (where as blood count, biochemistry, lipidemic profile, hs CRP, Fibrinogen, BNP) and the sST2 with the ELISA technique by using the Presage™ ST2 assay kit by Critical Diagnostics.
Results: Mean age of the total 106 subjects were at 56,45 ± 9,19 years, without any differences between the 2 groups. No difference was observed also at the sex distribution in the groups. The mean value of sST2 in group A (non diabetic controls) was 9,76 ± 5,21, statistically significant lesser than the 13,48 ± 5,75 of group B (diabetic patients) (p=0,017). There was no significant difference on the BNP levels among the 2 groups (group A: 26,08 ± 13,10, group B: 31,36 ± 31,85, p=0,507). Similarly, no statistically significant difference was noticed on the values of hsCRP, Fibrinogen, estimated GFR. In the analysis of all the population, it seems that sST2 value is affected by fasting glucose level (r=0,360, p=0.034), HbA1C (r=0.389, p=0.023) and HDL (r=0.304, p=0.048). The multivariable analysis accrues that HbA1C is an independent correlation factor to the sST2 (β=0.186, p=0.045).
Conclusion: No study until now has focused on the role of sSt2 on diabetic people. In our study we showed that diabetic people with preserved LV systolic function have higher levels of sST2 compared to non diabetic controls. This value seems to be affected by the level of glycemic control (HbA1c), fasting glucose and H.D.L. Further research needs to be done to uncover the underlying pathophysiological mechanisms.
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The impact of smoking cessation on metabolic factors in newly diagnosed patients with type 2 diabetes: a one-year prospective study
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Background and aims: Smoking is an independent risk factor for cardiovascular morbidity and mortality. Patients with type 2 diabetes-mellitus (T2DM) are at increased risk for cardiovascular events and smoking cessation is a priority for the prevention of macrovascular and microvascular complications. In the present prospective study we evaluated the impact of smoking cessation on glycaemic control and metabolic factors in subjects with newly-diagnosed T2DM.
Materials and methods: We recruited 193 smokers (96 male/97 female: age 57±7.8 years) with newly-diagnosed T2DM, without macrovascular complications, who were educated to smoking cessation, diet and exercise. A detailed history of smoking habits (years-frequency of smoking, types of tobacco used) was obtained. All subjects were contacted by phone every 2-weeks in the first 2-months and monthly thereafter with emphasis on smoking cessation. Ankle-brachial-pressure index (ABI) was measured by ultrasonography. Demographic, biochemical parameters, insulin-resistance and albumin-excretion-rate (AER) were measured at baseline and 1 year after smoking cessation.
Results: At baseline, smoking habit was associated significantly with younger age [OR, 95% C.I. 1.86 (1.79-1.94)], female gender [1.19 (1.04-1.96)], higher BMI [1.85 (1.73-2.00)], systolic-blood-pressure (SBP) [1.02 (1.00-1.05)], HbA1c [1.97 (1.05-1.99)] and insulin-resistance [1.62 (1.03-2.54)], dyslipidemia (low HDL-cholesterol and/or high triglyceride levels and /or high LDL-cholesterol) [1.96 (1.94-1.99)], higher AER [1.62 (1.00-1.90)] and lower ABI [0.005 (0.00-0.85)]. Marital status was associated with lower odds [0.09 (0.01-0.68)] whereas a higher education level with higher odds of smoking [3.80 (1.02-4.15)]. At the end of the 12-month period, 62.2% (n=120) of the studied population reported successful cessation. Pharmacological interventions for hyperglycaemia, dyslipidaemia and blood-pressure control were not different between the studied groups. Towards baseline, after adjustment for dietary-factors and exercise-level, smoking cessation had the highest contribution in the reduction of HbA1c [0.116 (0.081-0.158], insulin-resistance [0.184 (0.03-0.35)], dyslipidaemia [0.94 (0.82-0.99)], SBP [0.34 (0.32-0.43], AER [0.50 (0.23-0.72)] and increased ABI [0.001 (0.00-0.15)]. No significant differences were found between patients who continued smoking and those who quitted regarding BMI and waist-circumference. Microalbuminuria was reduced by 38% in subjects quitting smoking and by 16% in those who continued smoking (P<0.001).
Conclusion: Smoking cessation strategies are effective in subjects with T2DM. Smoking cessation improves glycaemic control and lipid profile and reduces blood-pressure and microalbuminuria. Stricter counselling and interventions for quitting smoking are warranted in patients with T2DM for the prevention of microvascular and macrovascular complications.

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Achievement of specified lipid and hs-CRP levels with ezetimibe/simvastatin vs atorvastatin in metabolic syndrome patients with and without atherosclerotic vascular disease
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Background and aims: Atherosclerotic vascular disease (AVD) and metabolic syndrome (MetS) are each associated with an increased risk of CHD. In-

tensive treatment of modifiable factors associated with AVD and MetS, such as dyslipidemia, is recommended by treatment guidelines, including achievement of specific LDL-C, non-HDL-C and Apo B levels. The aim of this *post hoc* analysis was to assess the proportion of patients with and without AVD treated with ezetimibe/simvastatin combination therapy versus atorvastatin reaching specified lipid and hs-CRP levels.

Materials and methods: Adult patients (N=1143) with MetS and hypercholesterolemia were randomized to ezetimibe/simvastatin combination tablet 10/20 or 10/40 mg or atorvastatin 10, 20, or 40 mg for 6 weeks. Prespecified dose comparisons were ezetimibe/simvastatin 10/20 mg vs atorvastatin 10 mg or 20 mg and ezetimibe/simvastatin 10/40 mg vs atorvastatin 40 mg.

Results: The rates and ratios of the predictive odds of achieving specified levels and 95% confidence intervals are presented in the table. Significantly more patients without AVD achieved the single LDL-C, non-HDL-C and Apo B levels and the combination of these three levels with ezetimibe/simvastatin vs atorvastatin for the specified dose comparisons, except in the ezetimibe/simvastatin 40 mg vs atorvastatin 40 mg dose comparison. Significantly more patients with AVD achieved the single LDL-C and non-HDL-C levels and the combined triple levels with ezetimibe simvastatin vs atorvastatin at all dose comparisons, and the single Apo B level only with the ezetimibe/simvastatin 10/20 mg vs atorvastatin 10 mg comparison. In both subgroups achievement of hs-CRP<2.0 mg/L was similar with both treatments at all dose comparisons.

Conclusions: Compared with atorvastatin at prespecified dose comparisons, treatment with ezetimibe/simvastatin combination resulted in significantly more MetS patients with or without AVD achieving most of the specified lipid levels and the combined lipid endpoints. Clinical benefit from reduction in cardiovascular outcomes through treatment with ezetimibe/simvastatin or through hs-CRP lowering has not been proven.

Table

Patients without AVD*	LDL-C<100 mg/dL	non-HDL-C <130 mg/dL	Apo B<90 mg/dL	Triple target†	hs-CRP<2.0 mg/L
E/S 20 vs A 10	90% vs 70%	87% vs 63%	65% vs 41%	65% vs 41%	46% vs 40%
OR‡ (95% CI)	3.76 (1.99, 7.12)	4.00 (2.23, 7.18)	2.72 (1.70, 4.35)	2.64 (1.65, 4.22)	1.31 (0.82, 2.07)
E/S 20 vs A 20	90% vs 76%	87% vs 73%	65% vs 49%	65% vs 49%	46% vs 39%
OR‡ (95% CI)	2.76 (1.44, 5.28)	2.50 (1.38, 4.55)	1.98 (1.25, 3.15)	1.92 (1.21, 3.06)	1.33 (0.84, 2.11)
E/S 40 vs A 40	92% vs 86%	90% vs 84%	75% vs 66%	75% vs 66%	42% vs 44%
OR‡ (95% CI)	2.00 (0.95, 4.20)	1.74 (0.87, 3.45)	1.49 (0.91, 2.44)	1.53 (0.94, 2.51)	0.92 (0.59, 1.44)
Patients with AVD*	**LDL-C<70 mg/dL**	**non-HDL-C <100 mg/dL**	**Apo B<80 mg/dL**	**Triple target§**	**hs-CRP<2.0 mg/L**
E/S 20 vs A 10	65% vs 39%	65% vs 39%	53% vs 29%	50% vs 24%	49% vs 52%
OR‡ (95% CI)	2.87 (1.43, 5.78)	2.87 (1.43, 5.78)	2.74 (1.33, 5.64)	3.13 (1.49, 6.61)	0.89 (0.45, 1.75)
E/S 20 vs A 20	65% vs 39%	65% vs 41%	53% vs 41%	50% vs 28%	49% vs 39%
OR‡ (95% CI)	2.88 (1.41, 5.89)	2.68 (1.32, 5.47)	1.59 (0.79, 3.21)	2.62 (1.25, 5.51)	1.46 (0.72, 2.98)
E/S 40 vs A 40	79% vs 46%	80% vs 49%	63% vs 48%	63% vs 40%	45% vs 41%
OR‡ (95% CI)	4.33 (1.97, 9.52)	4.21 (1.89, 9.40)	1.90 (0.92, 3.91)	2.63 (1.27, 5.44)	1.16 (0.57, 2.38)

A=atorvastatin; Apo=apolipoprotein; AVD=atherosclerotic vascular disease; CI=confidence interval; E/S=ezetimibe/simvastatin; hs-CRP=high sensitivity C-reactive protein; LDL-C=low-density lipoprotein cholesterol; non-HDL-C=non-high-density lipoprotein cholesterol; OR=odds ratio.

*Treatment comparisons are based on the logistic model with terms for treatment only

†LDL-C<100 and non-HDL-C<130 and Apo B<90 mg/dL

‡The ratio of the predictive odds of achieving the specified goal on E/S vs the comparison dose of A

§LDL-C<70 and non-HDL-C<100 and Apo B<80 mg/dL

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Statin therapy and serum transaminases among patients with type 2 diabetes and hepatitis C

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Background and aims: Statins are the most efficacious drugs for decreasing low-density lipoprotein cholesterol levels; they reduce both primary and secondary cardiovascular risk in the general population. The objective of this study was to determine the effect of statin therapy (atorvastatin) on serum aspartataminotransferase and alaninaminotransferese levels in patient with type 2 diabetes (T2DM) and hepatitis C. However, less is known about the safety of statin use in patients with liver disease.

Materials and methods: We selected 64 patients with type 2 diabetes mellitus and chronic hepatitis C who are treated with atorvastatin, 20 mg for 6 month. We evaluated body weight, blood pressure, liver enzymes, lipids, adipocytokines (adiponectin, leptin, resistin, TNFalfa, IL-6), insulin resistance (by Homeostasis model assessment - HOMA-IR) at baseline, 1 and 6 months. The liver fibrosis was non-invasively assessed using the Forns index; a value < 4.2 excludes liver fibrosis and a value > 6.9 is a predictor for significant fibrosis.

Results: Plasma triglycerides and cholesterol decreased (p<0.05), HDL-cholesterol and HOMA-IR increased (p<0.05), after 6 months. Aspartataminotransferase and alaninaminotransferese increased but we did not find significant statistically differences (median increased was 15.6 U/L for ALT and 7.2 U/L for AST). Forns index decreased at 6 months (p=0.048). Atorvastatin treatment had no effect on plasma adiponectin (p=0.569) but we observed reducing of leptin and resistin level (p=0.032 respectively p=0.0048). TNFalpha and IL-6 decreased but not significant statistically.

Conclusion: Among patients with hepatic C no significant elevation of liver enzymes during statin treatment was observed. Statin therapy should not be stopped or contraindicated in this patient population; however, more prospective clinical trials are needed to confirm the safety and efficacy.

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1262

Effect of nicotinic acid on combined hyperlipidaemia: A kinetic study on reverse cholesterol transport in humans

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Nicotinic acid improves the combined dyslipidemia frequently observed in patients with type 2 diabetes or metabolic syndrome. The highest treatment increase of HDL-C is observed with this drug but the underlying mechanisms are unclear. The aim of this study was to give further insights on the effect of nicotinic acid in humans by using a new dual stable isotope technique to estimate the cholesterol transport and metabolism in HDL and other lipoproteins. We recruited 8 patients with combined hyperlipidemia submitted to 8 week treatment with LAR nicotinic acid (2 g/d) associated with aspirin (300 mg/d). The patients were submitted before and at the end of the treatment to a dual infusion of 2H3 leucine and 13C2 acetate. The tracer/trace ratio was analyzed by mass spectrometry in Apo B100, Apo AI and cholesterol (free and esterified) in VLDL, IDL, LDL and HDL. The compartmental model to analyze the data was developed on SAAM II. With LAR nicotinic acid triglycerides declined (2.21±0.67 vs 1.19±0.62, p<0.05) as total cholesterol (2.15±0.47 vs 1.80±0.38, p<0.05) and LDL-C (1.28±0.45 vs 1.03±0.29, p<0.05) while HDL-C increased (0.41± 0.13 vs 0.49±0.10, p<0.05). There was no change on hepatic ApoB100-VLDL production but conversion rate of ApoB100-VLDL to ApoB100-LDL increased (0.091±0.039 h-1 vs 0.123h-1 ±0.039, p<0.05). There was no significant change on ApoAI-HDL kinetics. An increase of free cholesterol esterification was observed within HDL (0.13±0.045h-1 vs 0.23±0.13h-1, p<0,05)and an increase of esterified cholesterol catabolism in HDL (0.049± 0.014 vs 0.074± 0.036 mg.kg-1. h-1, p<0,09)associated with a decrease of esterified cholesterol transfer to VLDL and LDL(0.45± 0.11 vs 0.32± 0.14 h-1, p<0,05) through CETP. We concluded that LAR nicotinic acid decreased triglycerides by increasing vascular lipolysis and increased reverse cholesterol transport by increasing cholesterol esterification and catabolism

within HDL. The increase oh plasma HDL-C is mainly related to a decrease (30 %) of CETP cholesterol transfer.
Supported by: GSK

1263

Study of apparent aspirin resistance in 100 consecutive patients with type 2 diabetes in primary cardiovascular prevention

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Background and aims: Occurrence of aspirin resistance in type 2 diabetes (D2) is controversial. It has been considered as a cardiovascular risk factor whatever the underlying mechanism. However it is difficult to distinguish authentic platelet resistance to the inhibitory effect of acetyl salicylic acid from non compliance. The aim of this study was to establish the prevalence of aspirin resistance in type 2 diabetic patients in primary cardiovascular prevention, submitted to multiple therapies and consequently more likely to be inobservant.
Materials and methods: 100 consecutive aspirin treated D2 patients in primary cardiovascular prevention were included in an observational prospective mono centric study. Patients were 65±8 years old, had D2 for 16±8 years, HbA1c = 7.7±1.1%, and were treated by low dose of aspirin (75 - 325 mg /d, mean 120±6 mg/d). Platelet *a*ggregation was assessed by arachidonic acid (0.5 mg/ml) on platelet rich plasma. Normal platelet function was established in 55 D2 patients not treated by aspirin (CTRL), (57±11 years old, D2 for 11±7 years, HbA1c 7.6±1.2%). Blood was drawn in the fasting state, in the morning, before any treatment administration.
Results: Mean aggregation level in CTRL D2 was 81.3±13.7%. 83% of D2 patients treated by low dose of aspirin had an aggregation level below 60%, cut off corresponding to the lowest aggregation level found in CTRL D2. All these aspirin sensitive D2 patients had an aggregation level lower than 25% (mean 6.3±7.9%). In univariate analysis, no criteria (age, sex, duration of diabetes, micro-angiopathic complications, smoking, number of pills taken daily¼) was associated with the occurrence of unaltered aggregation function under aspirin treatment. In the 17 D2 patients with apparent resistance, 6 (35%) admitted to have omitted to take aspirin the day before assessment of platelet function. In the 11 remaining patients, 6 were tested again after giving special advice to take aspirin the day before. Platelet aggregation level was found below 25% in all these patients upon this second test.
Conclusion: In the present study, 17% of D2 patients in primary cardiovascular prevention had an apparent aspirin resistance. However the second test showing the expected platelet aggregation inhibition suggests that non compliance plays a major role in the occurrence of apparent resistance to aspirin.
Supported by: HCL

1264

Metformin use among patients with type 2 diabetes and renal impairment

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Background and aims: The most prescribed oral antidiabetic medication for treating type 2 diabetes (T2D), metformin is contraindicated in T2D patients with renal impairment due to risk of lactic acidosis.
Materials and methods: Metformin use among T2D patients with renal impairment was examined with a retrospective analysis of the I3 Innovus database from May 1, 2000 to December 31, 2008. T2D patients (ICD-9 codes 250.x0 or 250.x2) with renal impairment (serum creatinine concentration ≥1.5 mg/mL in males and ≥1.4 mg/mL in females) were identified. Stage of chronic kidney disease (CKD) was based on estimated glomerular filtration rate (eGFR), calculated from serum creatinine, age and gender (using MDRD formula). The index date was the same or later diagnosis date of T2D or CKD. The baseline period was 6 months prior to index date, and the follow-up period was 12 months after index date. Patients were 18 years or older and had at least one serum creatinine value and at least one claim for antidiabetic medications during the study period. Patients with type 1 diabetes were excluded.
Results: After applying all inclusion and exclusion criteria to 1.7 million T2D patients, a total of 1,985 patients were identified with CKD and T2D; the majority (76%) had stage 3 CKD (table). Mean age was 61 years, and two-thirds were male. In the follow-up period after diagnosis of T2D and renal impairment, 666 (34%) were prescribed metformin. Metformin use was highest in CKD stage 2, followed by stages 3 and 5 (table). Patients <65 years more often received metformin than those ≥65 years (37% vs. 27%, p<0.0001). Metformin use between female (34%) and male patients (33%) was similar (p=0.8175).
Conclusion: Metformin was maintained in T2D patients with varying degrees of renal impairment including severe kidney disease and kidney failure despite contraindications for use. Further studies are needed to investigate risk of lactic acidosis and glycemic control in this at-risk population.

Table:

Stages of CKD	Group 1: Patients with CKD and T2D (n=1,985)	Group 2: Patients in Group 1 on metformin [34% (666/1,985)]
2 mild (eGFR 60-89)	12% (235)	46% (108/235)
3 moderate (eGFR 30-59)	76% (1,517)	35% (525/1,517)
4 severe (eGFR 15-29)	9% (179)	9% (17/179)
5 renal failure (eGFR < 15)	3% (54)	30% (16/54)

Supported by: TPNA, Inc.

1265

Pioglitazone in addition to metformin improves erythrocyte deformability in patients with type 2 diabetes mellitus. Results from the PIOfix study

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Background and aims: Changes in hemorheology and blood viscosity were shown to contribute to the development of micro- and macrovascular complications. In patients with diabetes mellitus, increased hematocrit levels and reduced erythrocyte deformability are considered to impair microvascular blood flow and to reduce tissue oxygenation. In recent studies, treatment with pioglitazone was shown to improve endothelial function and to improve the overall risk for vascular complications in patients with type 2 diabetes mellitus. The aim of this study was to compare the effect of adding pioglitazone or glimepiride to metformin treatment on erythrocyte deformability in patients with type 2 diabetes mellitus (T2DM).
Materials and methods: This two arm, parallel study, covered 23 metformin treated T2DM patients (16 male, age: 57.2±10.7 years; BMI 32.7±4.3 kg/m^2) with an HbA1c above 6.5%. Patients were randomized to receive either 15 mg Pioglitazone (PIO) bid. or 1 mg Glimepiride (GLIM) bid. in combination with 850 mg Metformin bid. for 6 months. Blood samples were taken for the measurement of fasting glucose, HbA1c, fasting insulin, intact proinsulin, adiponectin, and heamatocrit (Hct). In addition, the erythrocyte elongation index (EI) was measured using laserdiffractoscopy (Rheodyn SSD, Myrenne GmbH, Roetgen, Germany) at a shear stress range from 0.3 Pa to 60 Pa.
Results: Both treatments significantly improved HbA1c levels (PIO -0.9±0.8%; GLIM -0.6±0.4%; p<0.05 respectively) and end up in comparable HbA1c levels after 6 months (PIO 6.5 ± 1.2; GLIM 6.2 ± 0.4) Treatment with PIO reduced fasting insulin (-8.2±15.1 mU/L; p=0.097), and intact proinsulin levels (-11.3±9.2 pmol/L; p<0.05), and increased adiponectin levels (8.1±4.6 µg/mL; p<0.05). Hkt slightly decreased during PIO treatment (-1.3±2.3%; p=0.09). No significant changes in these parameters could be observed during GLIM treatment. As shown table 1, PIO improved the EI, resulting in a significant improvement at all physiological shear stress ranges (0.6 to 6.0 PA). At a physiological shear stress rate of 1.2 Pa, the improvement in EI correlated with the increase in adiponectin levels (r=0.74; p<0.0001), and inversely with intact proinsulin levels (r=-0.47; p<0.05).
Conclusion: This is the first study showing an improvement in erythrocyte flexibility during treatment with pioglitazone which was correlated to an increase in adiponectin and a decrease in intact proinsulin levels, but independent from glycaemic control.

Change in erythrcyte elongation index (EI; * p<0.05 vs baseline; $ p<0.05 PIO vs. GLIM)								
Shear stress (PA)	0.30	0.60	1.20	3.00	6.00	12.00	30.00	60.00
GLIM (EI)	-0.4± 1.7	-0.5±1.1*	-1.1±2.5*	-1.5±3.1	-2.1±3.8	-1.3±2.9*	-1.3±3.5	-1.3±3.9
PIO (EI)	1.3±2.1	2.4±1.3*,$	3.2±2.2*,$	3.3±2.8*,$	3.1±2.9*,$	2.7±2.8$	2.5±2.6	2.7±2.6

Supported by: Takeda Pharma

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Examination of the cardiovascular safety of pramlintide with a meta-analysis of five controlled clinical trials in patients with type 2 diabetes mellitus

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Background and aims: Pramlintide was approved by the US FDA in 2005 as an adjunctive treatment for patients with diabetes who use mealtime insulin with or without oral antihyperglycaemic drugs who have not achieved desired glucose control. The FDA recently issued guidance on the type of evidence required to demonstrate that a new type 2 diabetes therapy is not associated with unacceptable cardiovascular risk. In the absence of guidance for currently marketed antidiabetic therapies, the cardiovascular risk of pramlintide was examined following the guidance for new therapies.
Materials and methods: Analysis of an integrated database of 5 randomised controlled type 2 diabetes trials (16 to 52 weeks) compared relative risk (RR) and hazard ratio (HR) of cardiovascular events with pramlintide vs a pooled comparator treated with either placebo or rapid-acting insulin. Background therapies included at least one type of insulin and in some cases oral antidiabetic agents. The primary endpoint was primary major adverse cardiovascular events (MACE) including cardiovascular mortality, myocardial infarction, stroke, acute coronary syndrome hospitalisation, and urgent revascularisation procedures. Subset MACE analysis used narrower criteria including only cardiovascular mortality, myocardial infarction, and stroke, while SMQ MACE included all Standardised MedDRA Query terms for these events. The broader secondary endpoint included the primary endpoint terms plus arrhythmia, heart failure, and mechanical-related events.
Results: The population included 1434 pramlintide and 582 pooled comparator subjects treated for a total of 960 and 359 patient-years, respectively. Demographics were comparable between groups (mean age 56-57 y, 52-54% M, BMI 32-33 kg/m^2, HbA_{1c} 9.0-9.1%). RRs and HRs between pramlintide and pooled comparator ranged from 0.86 to 1.11, depending on the endpoint and analysis method used. Corresponding 95% upper CI limits were below or close to the FDA-specified 1.8 threshold, suggesting no unacceptable increase in cardiovascular risk.

	Primary MACE		Subset MACE		SMQ MACE		Secondary CV	
	Ratio	(95% CI)	Ratio	(95% CI)	Ratio	(95% CI)	Ratio	(95% CI)
RR (Mantel-Haenszel)	0.86	(0.55, 1.34)	0.90	(0.46, 1.76)	1.10	(0.62, 1.96)	1.03	(0.74, 1.43)
HR (Cox)	0.88	(0.56, 1.38)	0.92	(0.47, 1.78)	1.11	(0.62, 1.99)	1.05	(0.74, 1.49)
HR (Andersen-Gill)	0.93	(0.62, 1.38)	0.95	(0.50, 1.81)	1.10	(0.64, 1.86)	0.92	(0.69, 1.21)

In addition, study of postmarketing reports has not revealed evidence of a signal for cardiovascular risk.
Conclusion: Based on these analyses, there appears to be no increased risk of cardiovascular adverse events associated with pramlintide treatment. However, interpretation of this type of analysis has the following caveats: these trials were not designed to assess cardiovascular outcomes, events were adjudicated retrospectively after unblinding, the number of cardiovascular events was small, and the duration of the trials was ≤52 weeks.
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Therapeutic effects of a selective estrogen receptor modulator (SERM) on bone and lipid metabolism in postmenopausal type 2 diabetic patients

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Background and aims: Diabetic patients are at risk of bone fracture due to poor bone quality, despite the preservation of bone mineral density, therefore management should include control of osteoporosis and vascular events as well as blood glucose. Raloxifene, a selective estrogen receptor modulator, prevents bone mass reduction and vertebral bone fractures in postmenopausal women, improves bone quality and prevents atherosclerosis and breast cancer. We investigated its effects on not only bone but also lipid, and glucose metabolism in postmenopausal patients with type 2 diabetes.
Materials and methods: Subjects were 144 female patients with type 2 diabetes who were younger than 80 years and have been menopausal for at least 2 year. The study was a randomized trial and subjects were assigned to one of 3 groups: No medication; 1 μg alfacalcidol daily; or 60 mg raloxifene daily. Outcomes were measured at baseline and at 6 and 12 months, including serum N-telopeptide (NTx), bone-specific alkaline phosphatase (BAP), homocysteine, TC, HDL-C, LDL-C, TG, HbA1c, fasting blood glucose, insulin, and HOMA-R. The primary outcome of the study was changes in LDL-C at 6 months and secondary outcome was serum NTx, BAP, homocysteine, and HbA1c at 6 and 12 months.
Results: Glucose metabolism was unchanged in all 3 groups. Regarding bone metabolism, both NTx and BAP were reduced at 6 months and further reduced at 12 months in the treatment groups (alfacalcidol group, p=0.001; raloxifene group, p=0.000, compared with baseline). These effects were most remarkable in the raloxifene group. Concerning lipid metabolism, LDL-C at 6 months, a major evaluation item, was significantly reduced in the raloxifene group only (p=0.029). Thus, this study meets the primary outcome by the treatment with raloxifen. HDL-C was elevated in the alfacalcidol group (p=0.007) whereas TG tended to decrease in the raloxifene group. Homocysteine, a bone quality marker, was significantly reduced in the raloxifene group at both time-points (p=0.001). There was no correlation between the rate of improvement of LDL-C and changes in markers of bone metabolism or of bone quality. Multivariate analysis identified TC, HDL-C, and the percent change of TG, but not markers of bone metabolism or quality, as significant determinants of the raloxifene-induced change in LDL-C.
Conclusion: Raloxifene improved lipid metabolism, especially significantly reduced LDL-C at 6 months after the treatment, and reduced homocysteine in postmenopausal type 2 diabetic patients. It has been suggested that this agent could improve defective crosslinks related to bone quality. In type 2 diabetic patients, poor bone quality is linked to increased risk of bone fracture. Since raloxifene improves bone quality as well as lipid metabolism, it is considered useful for all-round health care of postmenopausal type 2 diabetic patients.

PS 126 Peripheral and cerebral arteries

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Effects of early detection and intensive treatment on peripheral arterial disease - ADDITION Denmark

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Background: Peripheral arterial disease (PAD) is a marker of systemic atherosclerotic disease and an independent risk factor for foot ulcers and amputations. Measurement of ankle brachial index (ABI) is widely used in the diagnosis of PAD. People with diabetes have an increased risk of developing PAD. Prevalence among diabetic individuals aged over 50 years is estimated to be 29%. Evidence exists on treatment of these patients but there is no evidence on treatment or prevention of PAD in people with screen detected diabetes. Our aim was to study the effect of intensive multifactorial treatment and routine care on PAD in persons with screen detected diabetes in a cluster randomized controlled study.
Methods: We recruited 466 individuals with screen-detected diabetes identified through a screening programme in 87 general practices in the Danish arm of the ADDITION Study. Practices were randomized to intensive treatment (IT) or routine care (RC) before initiation of the screening programme. Individuals in the IT practices (45 practices) received a multi-factorial treatment programme including lifestyle advice (concerning diet, physical activity, medication adherence, and smoking cessation), prescription of aspirin and stepwise increases in pharmacological treatment of blood pressure, glucose and lipids according to strict targets. The RC group (42 practices) were offered treatment according to national guidelines. Anthropometric measures and blood samples were collected at baseline and at five year follow-up. At follow-up ABI was measured according to a standardized protocol. ABI was calculated using the highest blood pressure in each foot divided by the highest blood pressure in either arm. An ABI of <0.9 in either leg was considered "abnormal". A X^2 test was used to assess the hypothesis of no difference in prevalence of PAD between the IT and RC group at follow-up. Logistic regression, accounting for the cluster design, was used to calculate the odds of ABI<0.9 in the IT compared to the RC group.
Results: Baseline characteristics are shown in Table 1. The IT group had a prevalence of abnormal ABI in either leg of 7% (4; 10) compared to 9% (4; 13) in the RC group (p=0.43). The odds ratio of ABI<0.9 in IT compared to RC was 0.76 (0.38; 1.52), p=0.43.
Conclusion: The prevalence of PAD 5 years after a screen-detected diagnosis of diabetes was lower in the Danish arm of the ADDITION trial compared to cohorts of people with known diabetes. This could indicate that screening for diabetes identifies people at an earlier stage in the trajectory of PAD and/or that general diabetes prevention and treatment has improved significantly over the past decade. We did not find that intensive multi-factorial treatment in general practice led to a significant reduction in the prevalence of PAD compared to routine care as recommended by national guidelines.

Table 1 - Baseline Characteristics

	Routine	Intensive
No. of Patients	171	295
Male	57.9%	56.7%
Age (yr)	59.0 (7.0)	59.3 (6.6)
HbA1c	6.4 (1.1)	6.4 (1.4)
Systolic BP (mmHg)	145.1 (17.6)	144.4 (18.5)
Body Mass Index	30.4 (5.43)	30.3 (5.12)
Waist (cm)	103.1 (13.9)	103.3 (13.0)
Total Cholesterol (mmol/l)	5.82 (1.19)	5.64 (1.10)
HDL (mmol/l)	1.37 (0.32)	1.38 (0.37)
Triglycerides	1.7 (1.3)	1.6 (1.0)
Smoking Current	33.7%	31.3%

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The association between long term glycaemia and arterial stiffness is detectable with HbA_{1c} but not advanced glycation end-products in people with diabetes risk. The ADDITION-PRO study

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Background and aims: Increased arterial stiffness is one of the initial steps in the development of atherosclerosis. Long term exposure to hyperglycaemia is associated with arterial stiffness. Glycated proteins such as haemoglobin (HbA_{1c}) and advanced glycation end-products (AGE) reflect glycaemia during a longer period. HbA_{1c} is the frequently used assessment of long term exposure to glycaemia and is known to be associated with arterial stiffness. However, it is unknown whether the non-invasive assessment of AGE can detect the same association. We aimed to study how strongly HbA_{1c} and AGE are associated with arterial stiffness in a population at high diabetes risk.
Materials and methods: A Danish population at high diabetes risk originally identified through a stepwise screening programme was invited for a health examination in 2009 including assessments of HbA_{1c} and AGE by non-invasive skin autofluorescence. Plasma lipids, blood pressure, height, weight, and carotid-femoral pulse wave velocity (PWV) as an assessment of arterial stiffness were measured. Information of antidiabetic treatment and smoking was self-reported. Individuals on antidiabetic medication were excluded in the analysis. The effect of HbA_{1c} and AGE on PWV was assessed through linear regression models adjusted for age, sex, and pulse pressure, and furthermore adjusted for BMI, smoking, HDL cholesterol, and triglycerides. The standardized regression coefficients were compared.
Results: 378 individuals (mean age 66.2 years (SD: 6.2), 57% men, mean BMI 27.7 kg/m^2 (SD: 4.4), mean HbA_{1c} 5.9% (SD: 0.4), mean AGE 2.25 arbitrary units (SD:0.52)) are included in the analysis. HbA_{1c} was associated with PWV in the model adjusted for age, sex and pulse pressure (Table 1). This association remained significant after further adjustments. AGE was not associated with PWV at a statistically significant level.
Conclusion: Among individuals at high diabetes risk HbA_{1c} but not AGE measured by a non-invasive method is associated with arterial stiffness. These differences in associations could be explained by the high risk population, and that HbA_{1c} reflects a shorter period of glycaemia than AGE. The other explanation could be that HbA_{1c} is a more precise measurement of long term glycaemia than AGE measured by skin autofluorescence.

Table 1 Change in PWV (m/s) per 1 SD increase in the variable

	PWV (95% CI)		
	Model 1	Model 2	Model 3
HbA_{1c}	0.45 (0.25;0.65)	0.34 (0.13;0.55)	0.31 (0.10;0.52)
AGE	0.16 (-0.05;0.37)	0.10 (-0.11;0.30)	0.06 (-0.16;0.27)

Model 1: Adjustment for age, sex and pulse pressure
Model 2: Model 1 + BMI
Model 3: Model 2 + smoking, HDL cholesterol, and triglycerides

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Increased chemerin levels are associated with peripheral arterial occlusive disease and increased urinary albumin excretion rate in type 2 diabetic patients

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Background and aims: Chemerin is a recently discovered adipokine that regulates adipocyte differentiation and modulates chemotaxis and activation of dendritic cells and macrophages. Chemerin was found to be associated with obesity, insulin resistance and the metabolic syndrome. In addition, recent studies have demonstrated that chemerin has angiogenetic properties. Given the convergence of adipocyte and macrophage function, chemerin may provide an interesting link between obesity, inflammation and atherosclerosis in humans. Thus, the aim of our study was to determine whether

serum chemerin levels are associated with vascular disease and urinary albumin excretion rate in type 2 diabetic patients.
Materials and methods: Serum Chemerin levels were determined in 128 patients with Type 2 diabetes mellitus (T2DM) and different levels of albuminuria (54 with normoalbuminuria (NA), 45 with microalbuminuria and 29 with macroalbuminuria (MA)) as well as in 28 healthy control subjects (CO). Mean Age of the patients was 66±11 years and mean duration of diabetes was 13±8 years. Chemerin was measured by an ELISA (BioVendor, Heidelberg, Germany). Within the T2DM patients 32 patients had a history of peripheral arterial occlusive disease (PAOD), 26 with coronary heart disease, 11 with stroke and 57 with any cardiovascular disease (CVD).
Results: Circulating Chemerin levels are significantly elevated in diabetic patients compared to CO (240.7±77.5 vs 175.6±46.5 ng/ml, p<0.001). In the diabetic patients, serum chemerin levels are significantly associated with urinary albumin excretion rate (UAE, mg/24h; p=0.001). The highest chemerin levels were observed in patients presenting with MA (290.9±84.5 vs 223.1±53.9 ng/ml in NA, p<0.001). In univariate regression analysis of all quantitative variables chemerin was significantly associated with BMI (p=0.009), HbA1c (p=0.019), creatinine (p<0.001), estimated glomerular filtration rate (p=0.002), age (p<0.001) and UAE (p=0.001). The multivariate model revealed UAE (β=0.341, p=0.001), age (β=0.229, p=0.029) and BMI (β=0.231, p=0.028) as predictors of chemerin.Chemerin levels were significantly higher in patients with PAOD versus those without PAOD (264.6±74.9 vs. 217.0±72.5 ng/ml, p=0.002) and in those with any CVD vs those without (255.3±71.6 vs. 208.0±70.5 ng/ml, p<0.001), but did not differ between patients with or without history of myocardial infarction and stroke.
Conclusion: These are the first findings demonstrating that chemerin levels are significantly increased in T2DM patients presenting with PAOD, the most advanced vascular disease in diabetic patients, but not in those with a history of myocardial infarction or stroke. The strong relationship between chemerin and urinary albumin excretion rate could be of clinical relevance in particular since proangiogenic properties were recently found for this adipokine.

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Severe peripheral arterial obstructive disease predicts cardiovascular events in type 2 diabetes mellitus: Is there a gender difference?
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Background and aims: Peripheral Arterial Obstructive Disease (PAOD) predicts cardiovascular (CV) events both in the general and in the diabetic population: aim of the present study is to evaluate whether this predictive power is different in men and women affected by Type 2 diabetes mellitus (T2DM).
Materials and methods: We examined all Type 2 diabetic patients submitted to revascularization procedures (mainly percutaneous angioplasty) because of severe PAOD at our Unit from 1997 to 2009: 50 women and 87 men (identified as "Cases"). Each of them was associated with a control subject matched for gender, age, diabetes duration and lengths of follow-up at our Unit, but without PAOD (identified as "Controls"). In Cases and Controls, we evaluated: a)CV risk factors at the entry in the study (i.e., in Cases at the time of revascularization procedure and in Controls at a corresponding time period); b)the first fatal or non fatal coronary or cerebral vascular event and total and CV mortality during the follow-up. By Cox analysis, we calculated the Hazard Ratios (HR) for the first CV event, CV and total mortality.
Results: In Cases, women differed from men for age (73.8±7.5 vs 69.4±8.8 years, p< 0.0001) and diabetes duration (21.7±11.8 vs 16.4±10.0 years, p<0.0001); similarly in Controls (age 73.7±8.3 vs 69.6±8.3 years, p<0.0001, diabetes duration 21.4±11.8 vs 16.1±10.3 years, p<0.0001). Smoking habit (actual or previous) was present in 41.4% of men Controls vs 88.5% of men Cases and in 4% of women Controls vs 44% of women Cases. CV events occurred in 16/50 (32%) women Cases vs 7/50 (14%) women Controls, and in 33/87 (37.9%) men Cases vs 18/87 (20.7%) men Controls. CV mortality occurred in 16/50 (32%) women Cases vs 6/50 (12%) women Controls and in 26/87 (29.9%) men Cases vs 10/87 (11.5%) men Controls. Total mortality occurred in 23/50 (46%) women Cases vs 10/50 (20%) women Controls, and in 34/87 (39.1%) men Cases vs 17/87 (19.5%) men Controls. HR (and C.I.) of Cases vs Controls corrected for smoking habit were: a)for CV events, 3.177 (1.126-8.967, p=0.029) in women and 3.505 (1.666-7.373, p=0.001) in men; b)for CV mortality, 3.261 (1.169-9.094, p=0.024) in women and 3.303 (1.281-8.521, p=0.013) in men; c)for total mortality, 3.122 (1.352-7.210, p=0.008) in women and 2.818 (1.301-6.105, p=0.009) in men. After correction for HbA1c, serum creatinine, LDL-cholesterol, HDL-cholesterol, triglycerides and smoking habit, HR for CV events were 7.71 (2.101-28.289, p=0.002) in women and 3.513 (1.423-8.676, p=0.006) in men. HR conferred by male vs female gender adjusted for age, diabetes duration and smoking habit were: a) for CV events, 3.565 (1.222-10.403, p=0.020) in Controls and 0.867 (0.439-1.710) in Cases; b) for CV mortality, 3.752 (0.912-15.40, p=0.067) in Controls and 0.932 (0.448-1.939) in Cases; c) for total mortality, 3.080 (1.050-9.033, p=0.040) in Controls and 0.961 (0.501-1.843) in Cases.
Conclusion: In T2DM, PAOD requiring revascularization affects women at a more advanced age and after a longer diabetes duration: women are thus partly protected from this complication. However, when severe PAOD occurs, it completely effaces the CV protection conferred by female gender also in T2DM and predicts CV events, CV and total mortality with a comparable strength in the two genders.
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The association between serum osteoprotegerin levels with lower extremity arterial calcification in patients with type 2 diabetes mellitus
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Background and aims: Increased OPG levels have been found in diabetic patients with micro- and macrovascular complications. Recent studies have shown that serum osteoprotegerin (OPG) concentrations correlate with coronary artery calcification in patients with type 2 diabetes mellitus (T2DM). Lower extremity arterial calcification (LEAC) is common diabetes. However, no data exists on the association between serum OPG concentrations with LEAC in patients with T2DM. The aim of this study was to look for potential association between serum OPG levels and LEAC in patients with T2DM.
Materials and methods: A total of 74 patients (148 feet) with T2DM were recruited (mean age 67.8±9.0 years, duration of diabetes 15.3±10.9 years). In all patients radiographs were taken of both feet and ankles. LEAC was graded in a scale from 0-5 at 4 locations (posterior tibial and dorsalis pedis arteries bilaterally) as 0: absent; 1: barely visible; 2: slightly visible; 3: specific outline; 4: very dense equal to or lower than 2 cm; 5: very dense greater than 2 cm. The total LEAC score (0-20) at all 4 locations was calculated. Serum OPG levels were measured using ELISA. Diagnosis of peripheral arterial disease (PAD) was based on the presence of either biphasic, monophasic or blunted waveforms at the posterior tibial artery, while diagnosis of PN on neuropathy symptom score (NSS), neuropathy disability score (NDS) and vibration perception threshold (VPT).
Results: Patients with PAD (n=36) had significantly higher serum OPG levels in comparison with those without PAD (18.9±5.9 vs 14.2±3.9 pmol/l, p<0.001). Patients with PN (n=34) had also higher OPG levels than patients without PN (17.8±6.2 vs 14.9±4.0 pmol/l, p=0.021). Patients without or with less LEAC (grade 0-2 based on the maximum LEAC grade at one out of 4 locations; n=44) had lower OPG levels compared with patients with more severe LEAC (grade 3-5; n=30) (14.99±4.4 vs 18.61±6.0 pmol/l, p<0.001). The total LEAC score was significantly associated with age (r=0.23, p=0.011), pulse pressure (r=0.41, p<0.001), glomerular filtration rate (r= -0.20, p= 0.026), albumin-to-creatinine ratio (r=0.36, p<0.001), VPT (r=0.26, p=0.002), serum OPG levels (r= 0.25, p=0.004) and there was a trend for association with diabetes duration (r=0.17, p=0.055). The association between the total LEAC score and OPG concentrations remained significant after adjustment for age (p=0.035), GFR (p=0.021), diabetes duration (p=0.009) and PN status (p=0.003).
Conclusion: Serum OPG levels are increased in diabetic patients with PAD and are associated with severity of LEAC.

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Urinary D-lactate levels are increased in type 2 diabetic patients and are inversely associated with ankle arm index: The CODAM study

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Background and aims: Diabetes is associated with increased incidence of peripheral arterial disease (PAD) and its complications (ulcers, cardiovascular mortality). Intracellular glycosylation is increased in diabetes and is linked to vascular complications and may thus be involved in the increased risk for PAD in diabetes. Methylglyoxal is the key precursor for intracellular glycosylations. The glyoxalase pathway catalyzes the conversion of methylglyoxal to D-lactate. The aim of the present study was to investigate, first, whether urinary D-lactate, possibly a reflection of methylglyoxal, is increased in type 2 diabetes; second, whether D-lactate levels are associated with the presence of PAD; and third, whether this association is explained by intracellular hyperglycaemia.
Materials and methods: We investigated 510 (192 women, mean age: 59.5±7.0) participants of the Cohort on Diabetes and Atherosclerosis (CODAM) study with normal glucose metabolism (NGM; n=269), impaired glucose metabolism (IGM; n=114) and type 2 diabetes (DM2; n=127). Urinary D-lactate levels were measured with UPLC-MSMS and corrected for urine creatinine levels. Severity of PAD was determined by the ankle arm index (AAIx). We used linear regression analyses to investigate the association between D-lactate and AAIx, first with adjustments for sex, age and smoking and then for other risk factors (i.e. HDL, triglycerides, eGFR, mean arterial pressure and body mass index). We next determined whether the association could be explained by intracellular hyperglycaemia by further adjustment for HbA1c.
Results: We developed a new method for the detection of D-lactate with UPLC-MSMS with intra- and interassay coefficients of variation of 2.6% (n=10) and 5.6% (n=10) respectively. Median and interquartile range of D-lactate levels were 0.65 (0.34-1.39), 0.78 (0.37-1.51) and 1.39 (0.55-3.89) µmol/mmol creatinine for NGM, IGM and DM respectively (p for trend <0.001). AAIx *decreased* by 0.016 (95%CI: -0.027 to -0.005, p=0.005) per each standard deviation increase in D-lactate in analyses adjusted for age, sex and smoking, and by 0.014 (-0.020 to -0.003), p=0.013 after adjustments for other risk factors. After additional adjustment for HbA1c, this association was attenuated by 40%: to -0.010 (-0.022 to 0.001), p=0.077.
Conclusion: We found that higher urinary D-lactate, as a marker of intracellular methylglyoxal levels, is associated with PAD (as reflected by lower AAIx) in the CODAM study. This association was independent of potential confounders and other risk factors but could at least partially (40%) be explained by HbA1c, a marker for intracellular hyperglycaemia. Our results suggest that intracellular glycation may play an important role in the development of PAD.
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Evaluation and comparison of stroke neurological scales regarding long-term outcome of ischaemic stroke in diabetic patients

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Background and aims: Neurological impairment scales are frequently used to determine the neurological status of patients suffered ischemic stroke (IS). Stroke scales owe to be able to describe in detail the severity of neurological deficits and predict functional outcomes. The most common neurological impairment scales are: the National Institutes of Health Stroke Scale (NIHSS), the Orpington Prognostic Scale (OPS) and the Scandinavian Stroke Scale (SSS). The purpose of the present study is to evaluate and compare of NIHSS, OSS and SSS to the end points of (IS) - explicitly new stroke or death - in diabetic patients.
Materials and methods: We studied 212 diabetic patients [80(37.7%) men and 132(62.3%) women], 74.9±6 (SD) years old suffered IS. Acute stroke was defined according to World Health Organization criteria. The diagnosis of ischemic stroke was established by neurological examination and confirmed by computed tomography. Baseline neurological examination developed accordingly to NIHSS, OPS and SSS at admission to the hospital. End point was considered new incidence of stroke or death during one year. Evaluation of the linear relationship between NIHSS, OPS and SSS was calculated using Spearman's rank correlation coefficient. Logistic regression analysis models were conducted to investigate how accurate the neurological scales predict end points. The goodness of fit of the models was tested by Hosmer and Lemeshow Test and by Omnibus Test. Statistically significant values were considered for p<0.05.
Results: After 12 months 48(22.6%) diabetic patients [8(3.8%) men and 40(18.9%) women] have had new stroke or died. Analysis using Spearman's rank correlation coefficient indicates a statistically significant linear relationship between NIHSS and OPS (r=0.93, p<0.001). For these data mean±(SD) for NIHSS is 14.85±8.82 and for OPS 3.62±1.22. There was also significant correlation between NIHSS and SSS using Spearman's rank correlation coefficient (r=-0.88, p<0.001). For these data mean±(SD) for NIHSS is 14.85±8.82 and for SSS 35.26±12.2. OPS and SSS were also strongly correlated (r=-0.85, p<0.001). For these data mean±(SD) for OPS is 3.62±1.22 and for SSS 35.26±12.2. At logistic regression model baseline NHISS (RR=0.69 95% CI: 0.56-0.85, p=0.001) and SSS (RR=0.780 95% CI: 0.69-0.87, p<0.001) were significant predictors of new stroke or death, but OPS (RR=4.08 95% CI: 0.92-18.06, p=0.064) was not related to the end-points of IS in one year. For every one point increase in NIHSS, the relative risk of new stroke or death in diabetic patients increased by a factor of 0.69, while for every one point decrease in SSS, such risk increased by a factor of 0.78.
Conclusion: Neurological stroke scales can be used to predict likelihood of outcome in diabetic patients that suffered IS. NHISS, OPS and SSS are strongly correlated and a lot of the standardized assessments of each scale are comparable to each other. Baseline NIHSS and SSS are significant predictors of new stroke or death in diabetic patients in one year. SSS has a slightly higher prognostic capacity compared with NIHSS in diabetic patients. Further studies in larger population may reveal more interesting conclusions.

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Impact of different glycaemic indices during hospitalisation on long-term outcome of diabetic patients after an ischaemic stroke

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Background and aims: Diabetes is well recognized as a major risk factor for the development of stroke. It doubles the risk of ischemic stroke (IS) and worsens survival of patients with acute stroke. Hyperglycemia is prevalent in the early phase of acute ischemic stroke and is associated with worse neurological outcome and increased stroke mortality. The purpose of the present study is to assess the significance of a selection of admission and during hospitalization glycemic indices in the prediction of neurological outcome among diabetic patients that suffered IS.
Materials and methods: We studied 212 diabetic patients [80(37.7%) men 132(62.3%) women], 74.9±6 (SD) years old suffered IS for one year period time. Acute stroke was defined according to World Health Organization criteria. The diagnosis of ischemic stroke was established by neurological examination and confirmed by computed tomography. Neurological examination was developed according to the National Institute of Health Stroke Scale (NIHSS). Improvement of neurological outcome was considered a modification of equal or less than 4 points in NIHSS. Adjusted and unadjusted logistic regression analyses was conducted to investigate how accurate improvement in neurological outcome can be predicted by admission plasma glucose concentration (APG), fasting plasma glucose concentration (FPG), postprandial plasma glucose concentration (PPG), glucose spikes (GS), glycosylated hemoglobin (HbA1c) and diabetes duration in years for discharge, 3 months, 6 months and 12 months period time.
Results: At discharge neurological improvement observed on 32(15,1%) men and 44(20.8%) women [total 76(35.8%)], at 3 months 48(22.6%) men and 56(26.4%) women [total 104(49.1%)], at 6 months 72(34%) men and 76(35.8%) women [total 148(69.8%)] and at 12 months 72(34%) men

and 80(37.7%) women [total 152(71.7%)]. At discharge diabetes duration (OR=0.93 95% CI: 0.89-0.98, p=0.005) was significant predictor of outcome but APG, FPG, PPG, GS and HbA1c were not significant to functional outcome. At 3 months significant prediction of outcome was made by diabetes duration (OR=0.91 95% CI: 0.86-0.96, p=0.001) and HbA1c (OR =2.72, 95% CI: 1.27-5.83, p=0.01). At 6th month significant predictors of aggravation were diabetes duration (OR=1.08 95% CI: 1.01-1.15, p=0.012) and APG (OR=1.01 95%CI: 1.00-1.03, p=0.039). At 12th month APG (OR=1.01 95% CI: 1.00-1.03, p=0.018) and HbA1c (OR=1.76 95% CI: 1.06-2.91, p=0.027) predicted significantly the outcome.
Conclusion: Hyperglycemia worsens the neurological outcome of IS in diabetic patients. Each glycemic index corresponds to a significant predicting factor in different time period. Diabetes duration in addition to its recent prior regulation as expressed by HbA1c contribute critically to prognosis of IS in diabetic patients. Long diabetes duration predicts negative outcome of IS at discharge, 3rd and 6th month. HbA1c is a significant predictor of neurological outcome at 3 and 12 months. APG appears to have an important effect on the prognosis of IS at 6th and 12th month.

PS 127 Complications in type 1 diabetes

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Macrovascular complications may be associated with tighter glycaemic control in patients with type 1 diabetes: An analysis of primary care data in the UK

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Background and aims: The Diabetes Control and Complications Trial (DCCT) and follow-on Epidemiology of Diabetes Interventions and Complications Study (EDIC) conducted in the US and Canada demonstrated beneficial effects of tight glycemic control on the micro- and macrovascular complications of type 1 diabetes mellitus (T1DM) in a randomised controlled clinical trial setting. However, the outcomes of intensive glycemic control in clinical practice are not known. We conducted the present study to 1) identify and describe a cohort of patients with T1DM from the General Practice Research Database (GPRD) and 2) compare cumulative rates of micro- and macrovascular complications, based on the degree of HbA1c control achieved within 10 years of diagnosis.
Materials and methods: Data were derived from GPRD that collects data from medical practices within the UK. Logistic regression was used to create pairwise propensity scores between three groups of patients: tightly controlled (TC=HbA1c ≤6.5%); reasonably controlled (RC= HbA1c >6.5 and ≤7.5%); poorly controlled (PC=HbA1c >7.5%). Analyses of clinical endpoints utilized logistic regression with independent variables of propensity score quintiles, pairwise comparison of the HbA1c control group, and interaction of those two. Adjusted event rates were based on logistic regression models with all three groups included. Endpoints included microvascular complications (e.g. diabetic retinopathy, nephropathy) and macrovascular/cardiovascular (CV) events (e.g. myocardial infarction, ischemic heart disease, stroke). Endpoints were determined by diagnosis codes within the GPRD.
Results: The average age of study patients (N= 1086) at baseline was 25.8 (SD: 16.0) years; 60% male. Microvascular complications did not differ significantly between groups, although numerically the RC group demonstrated lower adjusted rates when compared to both the TC and PC groups (Table 1). There were no significant differences in macrovascular/CV events between either TC or PC patients when compared to RC, however a significant difference was noted after propensity score adjustment in the TC group compared to the PC group (OR=3.13; 95% CI, 1.39-7.14; p=0.006).
Conclusion: Using data from routine medical practice, we found, in contrast with the DCCT/EDIC results, no significant microvascular or CV benefit in T1DM patients treated intensively to control blood glucose versus those treated conventionally. Additionally, we observed increased macrovascular/CV events in TC versus PC patients. In light of results from recent high profile trials in type 2 diabetes mellitus which suggest increased mortality related to intensive glycemic control and its inherent risk of hypoglycemia, additional investigation of this association is warranted in T1DM patients.

Table 1: Microvascular Complications and Macrovascular/CV Events by Glycemic Control Groups

	Microvascular Complications			Macrovascular/CV Events		
	TC vs. RC	PC vs. RC	TC vs. PC	TC vs. RC	PC vs. RC	TC vs. PC
Odds Ratio	2.0	1.72	1.05	2.08	0.58	3.13
(95% CIs)	(0.75-5.26)	(0.97-3.07)	(0.53-2.13)	(0.79-5.56)	(0.29-0.13)	(1.39-7.14)
p-value	p=0.17	p=0.07	p=0.88	p=0.14	p=0.11	p=0.006
Adjusted Rates	16.9% / 9.6%	16.4% / 9.6%	16.9% / 16.4%	6.1% / 2.4%	2.7% / 2.4%	6.1% / 2.7%

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Tei Index identifies the early phase of left ventricular dysfunction in young patients with long-lasting type 1 diabetes mellitus and preserved systolic function

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Background and aims: The Tei Index (TI) is an easily obtained and reproducible Doppler parameter which reflects combined left ventricular (LV) systolic and diastolic function. Increased values of TI were found in patients with dilated cardiomyopathy and myocardial infarction and were proved to be an independent prognostic factor for higher mortality. The clinical usefulness of TI for young patients with type 1 diabetes mellitus (DM1) has not yet been fully studied. The aim of our study was an echocardiographic assessment of LV global function in patients with long-lasting DM 1, below 45 years of age, treated with intensive insulin therapy.
Materials and methods: The study group (DM): 100 pts (51F, 49M), mean age 30,0 yrs, with mean diabetes duration 15,7yrs, without drugs except insulin (mean 44 units/24h), with mean HbA1c 9,4%, without hypertension and overt heart disease, with good LV ejection fraction (>60%). The control group (C): 60 healthy persons (29F, 31M) in similar age. Mitral valve flow (MVF) and aortic valve flow spectra were registrated using pulse-wave Doppler. The TI, which is a ratio of the sum of isovolumetric contraction time and isovolumetric relaxation time (IVRT) to LV ejection time was calculated.
Results: There were no differences in mean values of heart rate, systolic and diastolic blood pressure, Body Mass Index, Body Surface Area, LV mass and LV mass index, lipids parameters and percentage of cigarette smokers between groups. In MVF assessment the mean E/A value (the ratio of early to atrial maximal velocity) was significantly lower in DM than in C group (1.33±0.3 vs 1.44±0.3, p=0.03), but it remained within the normal range for this age group. Mean TI value was especially significantly higher in DM than in C group (0.48±0.09 vs 0.38±0.05, p<0.001). In 51 pts of DM group TI exceeded the value of 0.49, the upper normal limit (mean value = 0.55 ± 0.05). All persons in C group had normal TI, with maximal value 0.46. The TI elevation is probably dependent on LV prolonged relaxation, due to the mean IVRT value was significantly higher in DM than in C group (60.1±8.0 vs 57.6±6.2 ms, p=0.03). There were no significant differences between the TI values related to duration and the level of metabolic control of DM1, number and type of complications, as well as between subgroups of women and men or younger and older patients. Correlation and multivariate analyses did not identify any important factor which might exert a significant effect on increased TI values in young diabetics.
Conclusion: Significantly higher Tei Index values observed in young patients with long-lasting type 1 diabetes mellitus with preserved LV systolic function may identify subjects with preclinical impairment of LV diastolic function. The significance of this phenomenon requires further investigation.

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Family history of hypertension or diabetes predicts IMT in well-controlled patients with type 1 diabetes

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Background and aims: In middle-aged subjects with type 1 diabetes, mortality due to ischemic heart disease is increased four- to seven-fold. Carotid intima-media thickness (IMT) is used as a surrogate marker of preclinical atherosclerosis. We searched for predictors of IMT in adults with type 1 diabetes in a prospective setting.
Materials and methods: A total of 57 patients (F/M = 27/30) were followed after their diagnosis until they reached 41±4 years of age, when duration of diabetes averaged 31±3 years. Cardiovascular risk markers [lipids, blood pressure, smoking, urinary albumin excretion rate (AER), alcohol consumption, family history] were evaluated at the 10 year, 20 year, and 30 year visits. During follow-up, IMT of the carotid arteries was determined.
Results: The patients were reasonably well-controlled with HbA1C of 7.7±0.9%, blood pressure of 133±16/78±9mmHg and LDL-Chol of 2.8±0.9mmol/L. Altogether 30 patients had positive family history of hypertension and/or diabetes (FHD+). These patients and those with FHD- did not differ concerning smoking, glycemia, lipids, AER, or BMI at any study visit. FHD+ patients had higher systolic blood pressure at the 20 year visit (129 ±16 vs. 117±11, p=0.042), despite having more antihypertensive medications. At any visit, other blood pressure values did not differ. At the 30 year visit, measures of IMT were significantly higher in FHD+ patients (eg. maximum IMT 1.03±0.12mm vs. 0.95±0.09mm, p=0.005) and their carotid plaque score was two-times higher compared to the FHD- group (p=0.005). Of the single measures from the 10 year visit, BMI showed the best correlation to the 30-year IMT (r=0.437, p<0.001).
Conclusion: Patients with type 1 diabetes and a positive family history of diabetes or hypertension have higher carotid IMT than patients without such a history. Even if well-controlled regarding risk factors, these patients are exposed to high cardiovascular risk.
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Association of risk factors and glycaemic control with endothelium-dependent vascular dysfunction in type 1 diabetes

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Background and aims: Endothelial dysfunction in patients with type 1 diabetes (T1DM) is an early event in the pathogenesis of vascular complications. However, little is known about the potential risk factors associated with impairment of the vascular reactivity in this population. The purpose of this study was to assess endothelial function in the microcirculation, correlating with possible factors involved such as glycemic control and potential markers of cardiovascular risk (uric acid and C reative protein- CRP) , and compare the results with those of healthy controls.
Materials and methods: A cross-sectional study was conducted in 57 T1DM patients , aged 32.5 (13-61) years and with a 15 (1-48) years disease duration, and 53 age-, sex-, and weight- matched controls. The median Hba1c was 9,3 (5,4-12,1).Skin perfusion was measured at the forearm using laser Doppler flowmetry during low-current iontophoresis of acethylcoline (ACh) (endothelium dependent response)and sodium nitroprusside(SNP)(Endothelium-independent response) . Post occlusive reactive hyperemia (PORH) and maximum vasodilator function during thermal hyperemia were also assessed. Diabetic patients underwent clinical and laboratory evaluation (disease duration, daily insulin dose, blood pressure, body mass index, urinary albumin excretion, lipid profile, glycemic control , uric acid and CRP).
Results: Microvascular response to ACh was significantly reduced in patients (p=0,002). However, despite the reduction of area under curve (AUC) of NPS, the analysis with repeated measures disclosed no difference between the groups in relation to the doses(p=0,15). Maximal skin microvascular vasodilation induced by thermal hyperemia was found to be higher in the control group than among patients {93,6 PU(perfusion units) (24,5-379-,9) and 56,6 PU (31,5-204,5)}, respectively p=0,004). On the other hand, during PORH, maximal increase in flux and AUC of hyperemic response did not differ between patients and controls, although the time frame to reach maximum flux and the time to half recovery after hyperemia was longer in patients than in controls (P=0.02). Endothelium-dependent response was correlated to diabetes duration (r= -0,33 p=0,01), tryglicerides (r= -0,37 p=0,005), insulin dose (r= -0,28 p=0,03), fasting glycemia (r= -0,3 p=0,02), hba1c (r= -0,34 p=0,001) and uric acid (r= -0,3 p=0,005), as well as independent-endothelium responses were correlated to capilar glycemia(r=0,30 p=0,02) and CRP (r=0,29 p=0,02). Uric acid levels were higher in non-diabetic than in diabetic subjects (4,40± 1,48 x 3,6 ± 1,0, respectively p=0,03), and were unrelated to endothelium-dependent response(r=0,08 p=0,55). In diabetics, on stepwise multivariate analysis, age, hba1c and uric acid were the most important factors associated with the AUC of ACh (p=0,02), and CRP with AUC of NPS (p=0,04).
Conclusion: We conclude that in T1DM patients the endothelium-dependent vascular response and maximal vasodilator capacity are significantly reduced and normal serum urate, glycemic control, age and CRP were the most important contributing factors to the variation of microvascular reactivity. And the inverse association of uric acid levels and ACh response can be explained probably by the loss of the antioxidant properties of urate.
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Impaired 24-hour blood pressure variation in adolescents and adult normoalbuminuric type 1 diabetes patients

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Background and aims: The absence of >=10% blood pressure (BP) drop at night ("non-dipping" phenomenon) is being recognized as a significant risk factor of vascular complications of diabetes. Little is known on the role of vascular factors like endothelium function and subclinical inflammation in the development of disturbed 24-hour blood pressure rhythm in type 1 diabetes (T1DM). The aim of the study was to assess blood pressure rhythm in adolescent and adult subjects with type 1 diabetes.
Materials and methods: The study comprised two groups of normoalbuminuric T1DM patients: Group A - 52 adolescents (mean age 14.1±3.0 yrs, diabetes duration 5.1±2.2 yrs, HbA1c 7.2±1.0%) with; Group B - 62 adults (mean age 34.1±7.2 yrs, diabetes duration 4.8±2.5 yrs, HbA1c 7.2±1.1%) and a group of healthy controls (Group C; mean age 23.1±4.4 yrs). All subjects had 24-hour blood pressure monitoring performed with the use of SpaceLabs 90207. Fasting plasma adhesion molecules sVCAM and sICAM, sE-selectin, adiponectin, interleukin IL-6, and TNF-alfa concentration were measured.
Results: 25 (48%) subjects from Group A and 9 (15%) from Group B were "non-dippers"; no "non-dippers" were found in Group C. There were no statistically significant differences in vascular and inflammatory parameters between "dippers" and "non-dippers" from Group A and Group B or the controls (table). However, mean 24-hour systolic (SBP) and diastolic blood pressure (DBP) was significantly lower in Group A than in Group B and C: 118±10 and 66±5; 126±11 and 73±6, and 128±12 and 73±6 mmHg ($p<0.01$). In particular, SBP at night was significantly lower in "non-dippers" than in "dippers" from Group A: 105±8 and 113±10 mmHg ($p<0.01$), while DBP was similar: 57±3 and 60±7 mmHg, respectively. There was a significant positive correlation between 24-hour SBP and body mass index (BMI) in Group A and B subjects ($r=0.41$ and $r=0.32$, respectively).
Conclusion: In adolescent or adult patients with T1DM non-dipping phenomenon is not associated with endothelial dysfunction or increased subclinical inflammation. In addition, unusually high prevalence of non-dipping in adolescent subjects with T1DM suggests that establishing this diagnosis with traditional criteria might not be at all appropriate in this population. Children and adolescents with T1DM display lower BP than the older persons, therefore they physiologically might not be subject to >=10% decrease of BP at night. For the assessment of diurnal blood pressure variation in adolescent T1DM patients different criteria of BP nighttime reduction might be necessary to apply.

	Gr. A		Gr. B		Gr. C
	dippers	non-dippers	dippers	non-dippers	
sVCAM (ng/ml)	1035±412	1039±537	1212±849	1025±832	924±428
sICAM (ng/ml)	370±129	410±170	427±153	399±293	354±164
sE-selectin (ng/ml)	43.4±15.9	40.8±25.3	42.1±15.9	46.0±22.3	38.3±29.1
Adiponectin (ng/ml)	13178±11439	11644±10441	12569±8638	11792±11222	13954±10319
TNF-alfa (pg/ml)	19.8±11.1	15.0±7.7	14.9±10.2	16.2±12.7	13.2±6.9
IL-6 (pg/ml)	4.9±4.7	6.7±10.2	5.9±4.2	6.8±6.5	6.2±5.7

Supported by: KBN

1281

Co-morbidity and survival of patients with type 1 diabetes on renal replacement therapy

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Background and aims: Mortality among patients with type 1 diabetes on renal replacement therapy is high. The effect of co-morbidities on their survival has not been previously estimated. The aim of this study was to estimate effect of co-morbidities on survival of type 1 diabetes patients on renal replacement therapy.
Materials and methods: An incident cohort of all type 1 diabetes patients entering chronic renal replacement therapy (n=656) in Finland between 2000 and 2008 was followed until death or the end of follow-up on 31 December 2008. All data were obtained from the Finnish Registry for Kidney Diseases, which collects information on co-morbidities at the start of renal replacement therapy. The main outcome was crude and adjusted relative risk of death according to co-morbidities.
Results: At start of renal replacement therapy 22% of the type 1 diabetic patients had coronary artery disease, 18% had peripheral vascular disease, 10% had cerebrovascular disease, 33% had left ventricular hypertrophy, and 7% had heart failure. All observed co-morbidities were significant predictors of death when analyzed univariably (RR 1.56-4.87). The 5-year survival probability of patients without (reported) co-morbidities was 74%, while it was 56% and 37%, respectively, for those with one or more than one co-morbidities. When the co-morbidities were studied in a multivariate model, also adjusting for age and gender, peripheral vascular disease (RR 1.88), left ventricular hypertrophy (RR 1.68) and heart failure (RR 2.50) remained independent risk factors of death.
Conclusion: Co-morbidities are common among type 1 diabetes patients entering renal replacement therapy, and they are strong predictors of death. Therefore, it is essential to diagnose and adequately treat co-morbidities.
Supported by: Diabetes Research Foundation

1282

Increased oxidative damage to de novo synthesized ApoA-1 in untreated type 1 diabetic patients - a novel method to identify the relative age of proteins

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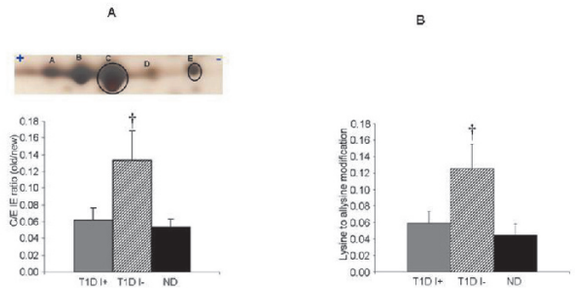
Background and aims: Oxidative damage and other post-translational modifications (PTM) in proteins, and accumulation of these proteins, may cause complications in diabetes. In order to address this issue, we developed a novel methodology to identify relative age of protein isoforms and to assess post-translational modifications by studying Apolipoprotein A-1.
Materials and methods: This method involves in vivo stable isotope labeling of proteins, protein isoform purification by two dimensional gel electrophoresis (2DGE), and isotopic enrichment (IE) and PTM analysis by tandem mass spectrometry. To label newly synthesized proteins, [ring-$^{13}C_6$]phenylalanine was intravenously infused for 8 hours in type 1 diabetic (T1D) participants with (I+) and without (I-) insulin treatment and non-diabetic (ND) participants (plasma glucose mmol/L; T1D I- = 17.0 ± 0.6, T1D I+ = 5.2 ± 0.2, ND = 4.9 ± 0.1).
Results: Only protein isoforms synthesized during infusion would incorporate the isotope label. In 2DGE and we identified 5 isoforms of ApoA-1. Pro-ApoA-1 (gel spot E) contained higher IE (ratio of [ring-$^{13}C_6$]phenylalanine to total phenylalanine), while older forms with higher degrees of damage (oxidation, deamidation) displayed less IE. Insulin deprivation in T1D increased the amount of oxidation in ApoA-1 ($P<0.05$) with more rapid maturation and damage of *de novo* synthesized pro-ApoA-1 ($P<0.05$) (% ^{13}C-labeling of mature ApoA-1 vs pro-ApoA-1: T1D I-= 13 ± 3, T1D I+ = 6 ± 1, ND = 5 ± 1).
Conclusion: The results show that ApoA-1, important for lipoprotein metabolism, is oxidatively damaged soon after synthesis during insulin deficiency that likely contributes to the cardiovascular complications during insulin deficiency and poor glycemic control in T1D.

Figure. A) The ratio of isotopic enrichment in older versus newer (C/E) ApoA-1 spot C demonstrates that the ratio is higher (P<0.05) in T1D during insulin deprivation indicating that newly synthesized ApoA-1 rapidly shifted to older form. B) Carbonylation (oxidation) in gel spot C of ApoA-1 is higher (P<0.05) in T1D during insulin deprivation.



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1283

The adverse effects of diabetic ketosis on the heart in young and middle-aged patients

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Background and aims: To observe the changes of myocardial enzymogram and electrocardiogram confirmed the myocardial damage during diabetic ketosis (DK)or diabetic ketoacidosis(DKA) in middle-aged patients.
Materials and methods: 78 hospitalized patients (45 male and 33 female) with DKA were recruited. Standard of Admission: Under 45 years old ; Above urine acetone bodies 2+; Without evidence of overt ischaemic heart disease; Without obvious infection and chronic disease history.Myocardial enzymogram which included creatine phosphokinase (CK), creatine phosphokinase isoenzyme(CK-MB) and aspart aminotransferases(AST) were measured on admission at ketosis stage and at 7 days after ketosis recovery. Electrocardiographs were also performed and compared during two stages.
Results: 78 hospitalized patients with DK or DKA had significantly higher levels of myocardial enzymogramCK,CK-MB and AST at ketosis stage compared to at stable stage. The changes of ECG were found in 46 cases (58.9%)of all the patients. The highest morbidity of ECG abnormality was ST-T change which was found in 32 cases(41.0%),ST segment depression in 8 cases, ST segment height in 1 case,T wave flat or inversion in 24 cases, sinus tachycardia in 18 cases (23.1%). 32 cases out of abnormal ECG in 46 cases improved at 7 day after ketosis recovery; in 20 cases recovered to normal. The levels of CK,CK-MB and AST with abnormal ECG at acute stage were significantly higher than those with normal ECG.. 3 patients with severe ketoacidosis in 78 cases, minute elevations of myocardial biomarkers 2 folds above reference value , ST segment depression 0.1mV in 2 cases and ST segment height in 1 case compatible with myocardial infarction. The higher levels of CK,CK-MBand AST restored to normal and change of ECG overtly improved at the stable stage, ST segment raised up 0.05 mV in 2 patient those ST segment depression,ST segment height in 1 case restored to normal also.
Conclusion: Diabetic ketoacidosis, particularly when severe, has a nonspecific myocardial injury, the level of myocardial enzymogram caused by diabetic ketosis could be abnormally higher. We should monitor the heart change and take an effective protective action during ketosis episode, the patients should be followed up and evaluated after recovery.

1284

Genetic variability of histone methyltransferases and the risk of diabetic complications in patients with type 1 diabetes

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Background and aims: There appears to be a complex interplay between genes and lifetime glucose exposure, which increases the risk of diabetic complications in patients with type 1 diabetes (T1D). However, the underlying molecular mechanism responsible for this phenomenon remains unexplained. We have demonstrated that exposure to glucose may lead to alterations of the methylation patterns of DNA and histones of essential genes, an epigenetic phenomenon that is increasingly considered to maintain the hyperglycaemic memory of critical cells. Since it has recently been shown that altered histone methylation is associated with an inflammatory phenotype in white blood cells and vascular cells when exposed to diabetic conditions, we hypothesized that genes coding for the enzymes that methylate histones, the histone methyltransferases SuV39 and SetD7, play a role in the development of diabetic complications.
Materials and methods: The first dataset included 2991 patients with T1D from the FinnDiane study. 811 patients had diabetic nephropathy (DN) defined as macroalbuminuria or ESRD, and 1070 patients had normal AER despite being exposed to diabetes for at least 15 years. 1168 patients were laser treated for retinopathy. The replication dataset included 888 patients from the Steno Diabetes Center, 452 were diagnosed with DN and 432 had normal AER. 416 patients had proliferative retinopathy. Genetic data on the CEPH population in the HapMap database (phase II) was used to select tagSNPs covering all SNPs in the genes *SuV39h1, SuV39h2* and *SetD7*. A total of 37 SNPs were selected for genotyping with the Sequenom MassARRAY iPLEX system or the TaqMan chemistry. P-values are presented both for raw data (p) and corrected for multiple testing (p_{corr}). Classical risk factors included in the logistic regression models are duration of T1D, systolic blood pressure, triglycerides, HbA_{1c}, male sex, smoking and BMI.
Results: In the FinnDiane, the SNP most strongly associated with DN was rs11100112 (*SetD7*) with minor allele frequencies of 18.0% in cases and 20.7% in controls (p=0.043/p_{corr}=NS). The minor allele of rs11100112 seemed protective after adjustment (OR=0.790[0.638-0.978], p=0.031). The SNP rs12572872 (near *SuV39h2*) showed a genotypic association with laser-treated retinopathy (p=0.0007/p_{corr}=0.030) with minor allele frequencies of 15.6% in cases and 18.7% in controls (p=0.003/p_{corr}=NS). When adjusted for classical risk factors, the minor allele containing genotype was protective (OR=0.814 [0.662-1.000], p=0.05). Six SNPs were chosen for replication but there were no significant associations, although the effects showed the same direction for both SNPs rs11100112 and rs12572872. When both study populations were combined (Fisher's method) no significant results were observed. When patients with HbA_{1c} and duration above/below medians were analyzed separately, no significant results were either recorded.
Conclusion: No clear association of polymorphisms in the specific histone methyltransferase genes examined and diabetic complications could be seen.

PS 128 Hypertension

1285

Initial combination therapy with aliskiren and hydrochlorothiazide is more effective than amlodipine in patients with stage 2 systolic hypertension and diabetes mellitus

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Background and aims: Agents that act on the renin-angiotensin-aldosterone system (RAAS) are considered drugs of choice for the treatment of hypertension in patients with diabetes mellitus. Direct renin inhibitors (DRIs) bind to and inhibit renin, which catalyzes the rate-limiting step of the RAAS. This 8-week, double-blind study compared the blood pressure lowering (BP) efficacy and safety of aliskiren/hydrochlorothiazide (ALI/HCT) combination *vs.* amlodipine (AML) in 860 male and female adults with stage 2 systolic hypertension (mean sitting systolic BP [msSBP] ≥ 160 and < 200 mmHg) and type 2 diabetes mellitus.
Materials and methods: After a 1-4 week washout, eligible patients (mean age: 59.8 years; mean BMI: 34.6 kg/m^2) were randomized to ALI/HCT 150/12.5 mg (n = 428) or AML 5 mg (n = 432). After 1 week, doses were force-titrated to ALI/HCT 300/25 mg or AML 10 mg, and patients were treated for an additional 7 weeks. The primary efficacy variable was change from baseline in msSBP at the Week 8 endpoint. Analysis to assess non-inferiority was performed, followed by superiority testing if ALI/HCT was shown to be non-inferior to AML.
Results: Baseline BPs were similar between the two groups: ALI/HCT: 168.0/91.4 mmHg and AML: 167.4/91.3 mmHg. At Week 8 endpoint, least squares (LS) mean reductions in msSBP were significantly greater with ALI/HCT *vs.* AML (-28.8 mmHg *vs.* -26.2 mmHg, LS mean difference: -2.6 mmHg; p <0.0001 non-inferiority; p = 0.0102 superiority). At study endpoint, a significantly greater percentage of patients receiving ALI/HCT achieved the BP goal of <130/80 mmHg compared with AML (23.2% *vs.* 13.8%; p <0.0001). Both treatments were generally well tolerated. Peripheral edema was more frequent with AML (16.2% *vs.* 2.1%) while dizziness was more frequent with ALI/HCT (3.0% *vs.* 0.9%).
Conclusion: Initial treatment with ALI/HCT 300/25 mg is significantly more effective than AML 10 mg at reducing msSBP and attaining BP control in patients with stage 2 systolic hypertension and type 2 diabetes mellitus.
Supported by: Novartis Pharmaceuticals Corporation

1286

Aliskiren reduces albuminuria independent of baseline blood pressure in combination with losartan in patients with type 2 diabetes and nephropathy

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Background and aims: Elevated blood pressure (BP) is a key contributor to development and progression of proteinuria and nephropathy in patients with type 2 diabetes. Direct renin inhibition with aliskiren (ALI) may offer renoprotective effects beyond BP reduction. This *post hoc* analysis assessed the influence of baseline BP on the antiproteinuric effect of ALI or placebo (PBO) added to losartan (LOS) in the Aliskiren in the EValuation of PrOteinuria In Diabetes (AVOID) study.
Materials and methods: In AVOID, 599 patients aged 18-85 years with hypertension and diabetic nephropathy received 6 months' ALI (150 mg force titrated to 300 mg after 3 months) or PBO added to LOS 100 mg daily and optimal antihypertensive therapy. Key exclusion criteria were non-diabetic kidney disease, eGFR <30 ml/min/1.73 m^2 and serum potassium >5.1 mmol/l. Changes in early morning urinary albumin:creatinine ratio (UACR) and eGFR at week 24 endpoint were assessed by subgroups of baseline BP: Group A, <130/80 mmHg (n = 159); Group B, <140/90 mmHg but >130/80 mmHg (n = 189) and Group C, >140/90 mmHg (n = 251).
Results: Mean baseline BP values (mmHg) for each subgroup were 120/71 (Group A), 133/78 (Group B) and 145/81 (Group C).The demographic, clinical and laboratory data were balanced between the three groups. The antiproteinuric effects of ALI were consistent across BP subgroups (19-22% reduction in UACR vs PBO, p = 0.977 for interaction). In Group C (BP >140/90 mmHg at baseline), the decline in eGFR was significantly lower with ALI than with PBO (p = 0.0096). There were no significant differences in the change in BP from baseline in any subgroup.
Conclusion: This *post hoc* analysis of the AVOID trial suggests that aliskiren 300 mg added to losartan 100 mg plus optimal antihypertensive therapy provides renoprotective effects independent of baseline BP in patients with type 2 diabetes and nephropathy.
Supported by: Novartis

1287

Patients with type 1 or type 2 diabetes have similarly increased pulsatility stress at comparable age of 50 years

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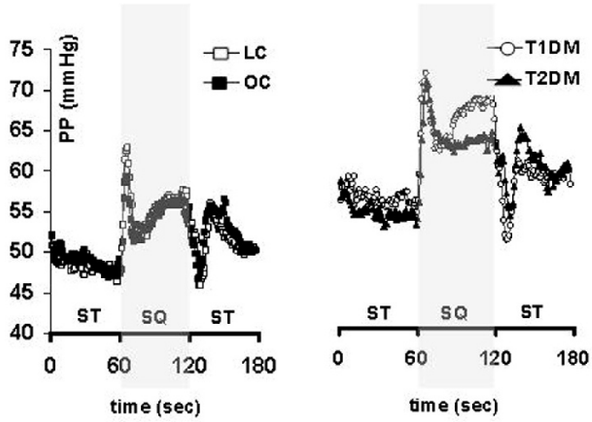
Background and aims: Arterial pulse pressure (PP) is considered as an independent cardiovascular risk factor in both patients with type 1 (T1DM) and type 2 (T2DM) diabetes mellitus. However, patients with each type of diabetes are exposed to a quite different toxic vascular environment. We compared PP and PP x heart rate (HR) double product (PPxHR = "pulsatile stress") during an active orthostatic test in patients with T1DM and patients with T2DM matched for age (40-60 years).
Materials and methods: 40 patients with T1DM (mean age 50 years, diabetes duration 23 years, body mass index - BMI - 23.0 kg/m^2, HbA1c 8.4%) were compared to 40 patients with T2DM (respectively, 50 years, 8 years, 29.7 kg/m^2, 7.6%). Patients taking antihypertensive agents or with renal insufficiency were excluded. All patients were evaluated with a continuous noninvasive arterial blood pressure monitoring (Finapres®) in standing (1 min), squatting (1 min) and standing position again (1 min). Patients with T1DM or T2DM were compared with two groups of 40 age- and BMI-matched healthy subjects (sex ratio 1/1 in all groups)(Figure).
Results: Despite similar mean arterial pressure (MAP), patients with T1DM and patients with T2DM showed significantly higher PP, HR and PPxHR double product levels than corresponding controls : in T1DM, 59 vs 52 mm Hg for PP (P= 0.016) and 5263 vs 4121 mmHg/min for PPxHR (P=0.0004); in T2DM, 58 vs 52 mm Hg for PP (P= 0.045) and 5359 vs 4321 mmHg/min for PPxHR (P=0.0023). However, there were no significant differences between patients with T1DM and T2DM regarding mean overall values of MAP (126 vs 128 mmHg), PP (59 vs 58 mmHg), HR (89 vs 88/min), and PPxHR product (5263 vs 5359 mmHg/min). During the transition from standing to squatting position, PP increase (+10 vs +8 mmHg) and HR reduction (-6 vs -6 beats/min) were significant but similar in both groups, resulting in a similar modest and non significant rise in PPxHR (+557 vs +449 mmHg/min).
Conclusion: Patients with T1DM have similarly increased PP, an indirect marker of arterial stiffness, and PPxHR double product, an index of pulsatile stress, as non-hypertensive patients with T2DM at similar mean age of 50 years. We hypothesize that such comparable pulsatile stress despite quite different natural history of the diabetic disease may be explained by a much longer exposure to chronic hyperglycaemia in patients with T1DM, on the one hand, and by the presence of associated risk factors such as obesity and insulin resistance in patients with T2DM, on the other hand.



PP changes during an orthostatic test :
standing (ST) - squatting (SQ : grey zone) - standing (ST)
Left : overweight controls (OC) versus lean controls (LC)
Right : patients with T1DM versus patients with T2DM

Supported by: Novo Nordisk Belgium

1288

Pulse pressure and systolic non-dipping, but not ambulatory arterial stiffness index, are independent predictors of macrovascular disease in patients with type 2 diabetes mellitus

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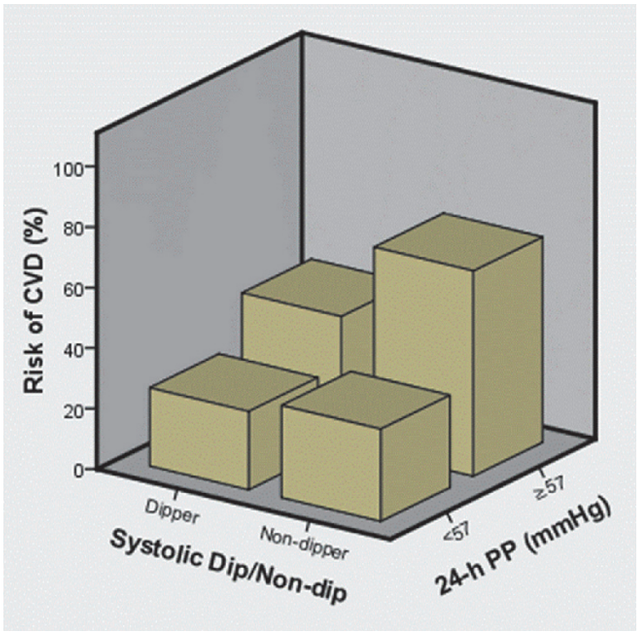
Background and aims: Patients with type 2 diabetes mellitus are at increased risk of cardiovascular disease (CVD). We examined the predictive ability of ambulatory blood pressure monitoring (ABPM) parameters for fatal and non-fatal CVD in patients with type 2 diabetes mellitus.
Material and methods: 108 patients with type 2 diabetes mellitus (mean duration 6.6 years) were followed for 9.5 (0.5-14.5) years. At baseline, all patients underwent ABPM.
Results: 45 patients experienced at least one CV event (35 non-fatal and 10 fatal). In bivariate analysis, CVD during follow-up was predicted by 24-h ambulatory pulse pressure (PP), ($p<0.01$), ambulatory arterial stiffness index (AASI), 24-h systolic blood pressure (BP) and systolic and diastolic non-dipping (defined as less than 10% nightly BP reduction) ($p<0.05$ for all). In Cox regression analysis with adjustment for established risk markers, 24-h PP and systolic night:day BP ratio both independently predicted CVD during follow-up: the adjusted hazards ratio (HR) for subsequent CVD per 10 mmHg increase in 24-h PP was 1.37 (95%CI 1.01;1.89, $p<0.05$), and the HR per 10 % increase in systolic night:day BP ratio was 1.54 (95CI 1.03; 2.30, $p<0.05$). Only 8 out of 31 (26%) patients with both 24-PP below the median (57 mmHg) and normal nocturnal systolic BP dipping pattern had incident CVD during follow up, whereas 19 out of 28 patients (68%) with both 24-h PP above or equal to the median and systolic non-dipping had incident CVD ($p<0.01$), figure 1.
Conclusion: 24-h PP and impaired nocturnal systolic BP decline, but not AASI, were independent predictors for CVD in type 2 diabetes patients.
Figure 1:



Supported by: Central Region Denmark Research Fund

1289

Mechanisms of effect of weight loss on blood pressure

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Weight loss has a clear blood pressure-lowering effect, the pathophysiological mechanisms are not completely understood. The aim of this study was to evaluate the effect of significant weight loss on blood pressure (BP) and its pathological mechanisms.
Patients and methods: Patients with documented hypertension who underwent laparoscopic gastric bypass were studied. Antihypertensive treatment was withdrawn one week before the evaluation. Anthropometric, BP (24-h ambulatory BP measurement) assessment, and blood samples for renin-angiotensin-aldosterone system [RAAS, plasma renin activity (PRA), aldosterone, angiotensin II and angiotensin converting enzyme] and sympathetic nervous system (metanephrine, normetanephrine and noradrenaline) analysis were performed before surgery at 4 and at 12 months postoperatively.
Results: Eighteen patients were studied, 12 females, with 50.8 (38-63) years old, with hypertension duration of 6.4 (1-20) years and excess body weight of 55.6 (36-73) kg. Twelve months after surgery: the BMI decreased from 45.6 to 32.1 Kg/m^2; excess body weight loss was -37.2 Kg; 13 (72%) patients had completed resolution of hypertension while 5 (28%) patients had improvement; 24-h (systolic -18.8/diastolic -7-7 mmHg), daytime and night-time BP values decreased significantly. The PRA (0.30 to 0.21 ng/mL*h), aldosterone (5.3 to 3.1 ng/dl) and noradrenaline (121.5 to 77.5 pg/mL) also had a significant decrease.
Conclusion: Weight loss is associated with reduction of the BP, RAAS and sympathetic system in obese hypertensive subjects.

1290

Assessment of pulsepen for detection of pseudohypertension in diabetic patients

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Background and aims: In the diabetic population, hypertension is often severe and resistant despite the combination of several antihypertensive drugs. Stiffness of brachial arteries may induce an overestimation of intra-arterial

blood pressure (BP) called pseudohypertension. Evidence for pseudohypertension is difficult to establish in clinical practice. A new non invasive method (Pulsepen) based on the estimation of central aortic pressure by applanation tonometry has been proposed for this purpose but was not previously assessed in diabetic patients.
Materials and methods: To compare Pulsepen and Dinamap versus intra-arterial pressure (method of reference) and to assess the capacity of Pulsepen to screen pseudohypertension in diabetic patients with resistant hypertension, BP was simultaneously recorded in diabetic patients by the three methods during an endovascular procedure (arteriography or coronarography).
Results: Our study population included 11 type 2 diabetic patients (7 men and 4 women), aged 66.5±6.9 years, with a duration of diabetes of 23.7±5.4 years, a mean BMI of 31.5±5.5 kg/m^{2}and a mean HbA1c level of 8.5±1.5%, with persistent hypertension despite two antihypertensive drugs at the time of inclusion. Seven patients were in secondary prevention of cardiovascular disease. Systolic BP (SBP) was significantly underestimated by: - 10.6± 8.24 % with Pulsepen (p=0.009) and by -4.63± 6.89 % with Dinamap (p=0.04). Diastolic BP (DBP) was insignificantly overestimated of +11.7±19.5 % (p=0.09) and by +15.3±19.9 % (p=0.06), respectively. The regression coefficients versus intra-arterial values were: SBP r^2 0.82 (p=0.0001), DBP r^2 0.64 (p=0.002) for Pulsepen and SBP r^2 0.87 (p<0.001), DBP r^2 0.65 (p=0.002) for Dinamap. According to pseudohypertension determined from the dinamap measurements, one patient had systolic pseudohypertension and two patients diastolic pseudohypertension. The Pulsepen was unable to detect the 3 cases of pseudohypertension.
Conclusion: Our data suggest that Pulsepen is not a reliable method in order to estimate intraarterial BP and to detect pseudohypertension in diabetic patients with resistant hypertension. This study underlies the difficulties to accurately measure BP in diabetic patients.

Systolic, Diastolic, Mean, Pulse blood pressure differences

	Pulsepen BP-intraarterial BP (mmHg)	p	Dinamap BP-Intraarterial BP (mmHg)	p
SBP	- 18.8 ± 15.5	0.009	- 8.7 ± 12.3	0.04
DBP	+ 7.6 ± 15.1	0.09	+ 10.1± 15.4	0.06
Mean BP	- 3.2 ± 10.7	0.26	+ 3.8 ± 9.9	0.09
Pulse BP	- 28.6 ± 24.7	0.03	- 18.8 ± 22.3	0.004

1291

A proteomic approach to the long-term experimental hypertensive-diabetic myocardium

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Background and aims: Diabetes is a worldwide pandemic that may damage the heart. Myocardial protein alteration has been described in short-term injury. However there is not information about the chronic pathology and its association with hypertension.
Aim: To analyze the myocardial proteome and molecular pathways of long-term diabetic/hypertensive rats.
Materials and methods: Normotensive and spontaneously hypertensive (SHR) rats received either streptozotocin or vehicle. After 22 weeks, type-I diabetic (DM1), SHR, DM1/SHR and control rats were sacrificed and the left ventricles studied by a proteomic approach based on DIGE and MALDI-MS. Differential proteins were validated by immuno-histochemistry, Western blot and Electrophoretic Mobility Shift assay (EMSA) and clustered into different biochemical pathways using bioinformatics tools.
Results: Long-term DM1 and hypertensive hearts are characterized by hypertrophy, fibrosis and apoptosis. By proteomics, we found 29, 55 and 58 proteins altered in the myocardium of long-term DM1, SHR and DM1/SHR rats, respectively (ratio≥ 1.35-fold with p≤0.05). Among these, DM1 myocardium presented over-expression of cytoskeleton and apoptosis proteins and down-regulation of anti-apoptotic and mitochondrial metabolic enzymes. In SHR and DM1/SHR rats these changes were exacerbated and, in addition, fatty acid ß-oxidation enzymes were decreased. Other enzymes for amino acid and alcohol metabolism were also altered. Bioinformatics suggested the implication of specific cytoskeleton factors, cytokines and fatty acid nuclear receptors in these pathologies. Peroxisome-activated receptors (PPARs) were stimulated whereas hepatocyte nuclear factor-4α receptor (HNF-4α) was not.
Conclusion: Long-term DM1 and markedly SHR and DM1/SHR hearts presented altered proteomes. Down-regulation of fatty acid and carbohydrate metabolisms and up-regulation of hypertrophic and apoptotic proteins may contribute to the hypertensive and diabetic-hypertensive cardiomyopathy. New unveiled factors as fatty acid nuclear receptors may be used as potential therapeutic targets for these diseases.
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1292

In subjects with type 2 diabetes, liraglutide, a once-daily human GLP-1 analogue, reduces systolic blood pressure with negligible impact from weight loss

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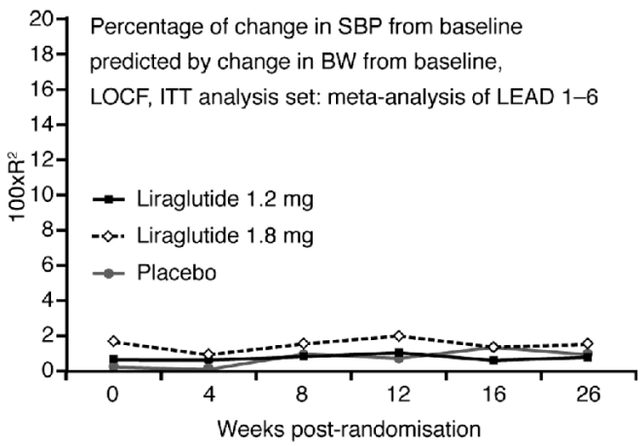
Background and aims: Hypertension is a major risk factor for myocardial infarction and stroke, and is more common in individuals with type 2 diabetes (T2D) than in the general population. Modest weight loss can result in significant long-term reductions in blood pressure and thereby reduce the risk for hypertension. In phase 3 clinical trials, in addition to improving glycated haemoglobin (HbA_{1c}) by 1.0-1.5%, liraglutide also improved systolic blood pressure (SBP) by 2-7 mmHg and produced sustained body weight (BW) reductions of 2-3 kg. However, the specific nature of the relationship between the effect of liraglutide on SBP and BW is not well-characterised.
Materials and methods: A meta-analysis of six randomised phase 3 clinical trials (n=3967) was performed in the liraglutide (1.2 and 1.8 mg) and placebo arms to investigate the relationship between changes in SBP and BW from baseline at each post-randomisation visit up to 26 weeks using an analysis of covariance (ANCOVA) model with treatment, trial, previous OAD treatment as fixed effects, country as random effect, and change in BW from baseline as covariate. The percentage of the change in SBP predicted by the change in BW is given by the 100 × R^2 (the square of the Pearson correlation coefficient), a goodness-of-fit index. The closer the R^2 is to 1, the stronger the relationship between change in SBP and BW.
Results: The analyses show a consistent trend of a very weak correlation between change in SBP and change in BW over time. At Week 2, up to 2% of the change in SBP could be predicted by the change in BW for both liraglutide doses and placebo. At Week 26, both liraglutide arms and placebo had a percentage of R^2 below 2%. The Pearson correlation between the change in SBP and the predicted change in SBP obtained from the ANCOVA model was also very weak with R^2 below 4%.
Conclusion: A minimal amount of the observed effect of liraglutide on change in SBP is predicted by change in BW. As a result, the effect of liraglutide on SBP cannot be explained by weight loss alone. In order to better understand the effect of liraglutide on SBP, mechanistic studies are warranted.



Supported by: Novo Nordisk

1293

The prevalence and severity of hypertension in patients with type 2 diabetes of Yemenite origin is lower than in non-Yemenite diabetics

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Background and aims: Yemenite Jews who immigrated to Israel during the years 1948-1950 had a very low prevalence of diabetes (0.05%) which increased to 12% within 40 years in parallel with their lifestyle changes. However, previous limited data suggested that the prevalence of hypertension (HTN) in Yemenite Jews remained lower than the prevalence in the general Israeli population. The aims of the current study were to determine in a group of patients with type 2 diabetes of Yemenite (Y-DM) or non-Yemenite (NY-DM) origin the prevalence of HTN and lifestyle patterns including their adherence to the Dietary Approaches to Stop Hypertension (DASH) diet.
Materials and methods: A cross-sectional study of 63 Y-DM patients (2 parents of Yemenite origin) and 120 NY-DM patients (neither parent of Yemenite origin) was conducted at a Diabetes Clinic. Medical and lifestyle information was collected including food frequency questionnaire (FFQ).
Results: The age and sex distribution were similar in the Y-DM and NY-DM groups (63 ± 7 years vs. 64 ± 7 years; 57% males vs. 56% males, respectively). The duration of diabetes in the Y-DM group was 16 ± 10 years vs. 13 ± 9 years in the NY-DM (P = 0.07) and the Y-DM group had lower weight and waist circumference (72 ± 13 kg vs. 85 ± 17 kg and 95 ± 11 cm vs. 105 ± 13 cm, P < 0.001). The prevalence of HTN was significantly lower in the Y-DM group compared with the NY-DM group (63% vs. 83%, P= 0.003). Despite having similar blood pressure control, patients in the Y-DM group necessitated less blood pressure medications than patients in the NY-DM (1.6 ± 1.8 vs. 2.5 ± 1.7, P= 0.002). In addition, in only 30% of the Y-DM group HTN was diagnosed before or concomitantly with the diagnosis of diabetes compared with 51% in the NY-DM (P= 0.035). In a logistic regression analysis, Non-Yemenite origin was independently associated with a higher prevalence of HTN (odd ratio 3.0, 95% CI :1.5-6.3, P= 0.0025). There were no significant differences between the 2 groups in physical activity, total calories consumed and the DASH score (30 ± 3 vs. 31 ± 3).
Conclusion: Despite the marked increase in the prevalence of type 2 diabetes in Yemenite Jews who immigrated to Israel, the prevalence of HTN in diabetics of Yemenite origin remained significantly lower compared with Non-Yemenite diabetics and blood pressure control necessitated less medications. These results are not due to differences in life style including adherence to the DASH diet and other mechanisms have to be explored.

PS 129 Dyslipidaemia and lipoproteins

1294

Colesevelam for Hispanic patients with hypercholesterolaemia and prediabetes

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Background and aims: Both hypercholesterolemia and prediabetes increase cardiovascular disease risk. Colesevelam has been shown to reduce LDL-C and fasting plasma glucose (FPG) in patients (pts) with hypercholesterolemia and prediabetes. A 16-week (wk), randomized, double-blind, placebo-controlled, multinational study evaluated the lipid- and glucose-lowering effect of colesevelam in pts with hypercholesterolemia and prediabetes.
Materials and methods: This post-hoc analysis evaluated the effect of colesevelam in a subpopulation of Hispanic pts (those self identified enrolled in Colombia, Mexico, and the US) included in the 16-wk study. Adults with untreated prediabetes (2-h post-OGTT glucose: 7.8-11.1 mmol/L and/or FPG: 6.1-6.9 mmol/L), LDL-C ≥2.6 mmol/L, and triglycerides (TG) <5.7 mmol/L were randomized 1:1 to colesevelam 3.75 g/d or placebo. Primary endpoint was percent change in LDL-C from baseline to Wk 16. All efficacy analyses were performed using last observation carried forward at Wk 16.
Results: In total, 153 Hispanic pts received colesevelam (n=77) or placebo (n=76). There was a significant LS mean percent change with colesevelam vs placebo from baseline to Wk 16 in: LDL-C (-14.7% vs 4.8%; treatment difference [TD]: -19.4%; *P*<0.0001), non-HDL-C (-10.2% vs 1.8%; TD: -12.0%; *P*<0.0001), total cholesterol (-6.9% vs 2.8%; TD: -9.7%; *P*<0.0001), apoB (-8.0% vs 2.2%; TD: -10.2%; *P*=0.0002), and TG (median: 5.9% vs -8.1%; TD: 15.4%; *P*=0.003). ApoA-I increased with both colesevelam (5.0%) and placebo (4.4%) at Wk 16. A significantly greater proportion of pts achieved LDL-C <2.6 mmol/L with colesevelam vs placebo at Wk 16 (27% vs 11%; *P*=0.002). Colesevelam vs placebo also produced significantly greater reductions from baseline in FPG (median: -0.2 vs -0.1 mmol/L; TD: -0.1 mmol/L; *P*=0.024) and HbA_{1c} (mean: -0.14% vs -0.02%; TD: -0.12%; *P*=0.009) at Wk 16. The proportion of pts achieving normalization of glucose (FPG <5.6 mmol/L) was significantly greater with colesevelam vs placebo (44% vs 23%; *P*=0.0497). Overall, colesevelam was well tolerated in Hispanic pts with hypercholesterolemia and prediabetes (Table).
Conclusion: In Hispanic pts with hypercholesterolemia and prediabetes, colesevelam may be a suitable option for lowering LDL-C and improving the metabolic profile. Further investigation regarding the effect of colesevelam on progression to type 2 diabetes is warranted.

Summary of Adverse Events		
n (%)	Colesevelam (n=81)	Placebo (n=78)
Patients reporting AEs	44 (54)	46 (59)
Patients reporting drug-related AEs	13 (16)	10 (13)
Patients reporting SAEs	1 (1)	2 (3)
AEs occurring in ≥5% of patients		
Back pain	3 (4)	5 (6)
Constipation	9 (11)	3 (4)
Diarrhea	4 (5)	6 (8)
Headache	6 (7)	10 (13)
Hypertension	1 (1)	5 (6)
Influenza	9 (11)	7 (9)

Supported by: Daiichi Sankyo, Inc., who markets colesevelam in the US

1295

Meta-analysis evaluating the proportions of patients with and without diabetes achieving lipid/lipoprotein goals with ezetimibe/statin combination therapy versus statin alone

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Background and aims: Treatment guidelines identify low-density lipoprotein cholesterol (LDL-C) as a major target of treatment in hypercholesterolemic patients with non-high-density lipoprotein cholesterol (non-HDL-C), apolipoprotein (apo) B and high-sensitivity C-reactive protein (hs-CRP) as secondary targets or emerging risk factors. This meta-analysis compared proportions of hypercholesterolemic patients with and without diabetes achieving various targets specified levels following treatment with ezetimibe 10 mg plus statin therapy (pooled across statin dose/type; EZE/statin) versus statin monotherapy (pooled across statin dose/type).
Materials and methods: This was a pooled analysis of 27 previously published, randomized, double-blind, active or placebo-controlled clinical trials conducted in 21794 adult patients (age range: 18-81 yr) with elevated LDL-C (LDL-C range: 1.81-6.48 mmol/L) receiving EZE/statin or statin alone for 4-24 wk. Patients were classified as having diabetes (either type 1 or 2). This analysis evaluated % of patients achieving various treatment goals at study endpoint in patients with (n=6541) and without diabetes (n=15253). Adjusted odds ratios (95% CIs) were calculated to evaluate the between-treatment ability to achieve specified levels in the overall population and within subgroups.
Results: Calculated odds ratios for the single levels are presented in the Table. Significantly more patients with and without diabetes achieved LDL-C (<2.59, <1.99, and <1.81 mmol/L), non-HDL-C (<3.37 and <2.59 mmol/L), apo B (<0.9 and <0.80 g/L) and hs-CRP (<2 and <1 mg/L) levels with EZE/statin vs statin alone. Patients with diabetes were more likely to reach single LDL-C and apo B targets than were non-diabetes patients. Greater goal attainment rates with EZE/statin vs statin alone also were seen for the dual (LDL <2.59 mmol/L and apo B <0.9 g/dL, LDL-C <1.81 mmol/L and apo B <0.8 g/L, LDL-C <1.99 mmol/L and apo B <0.8 g/L as well as hs-CRP <2 mg/L and LDL-C <2.59 mmol/L) and triple targets (LDL-C <2.59 mmol/L and apo B <0.9 g/L and non-HDL-C <3.37 mmol/L as well as LDL-C <1.81 mmol/L and apo B <0.8 g/L and non-HDL-C <2.59 mmol/L) both in the overall population and by diabetes status.
Conclusion: In this meta-analysis, significantly more patients with and without diabetes achieved specified LDL-C, non-HDL-C, apo B and hs-CRP levels with treatment with EZE/statin combination therapy vs statin monotherapy. The goal attainment for LDL-C, non-HDL-C and Apo B were significantly greater in patients with diabetes than those without diabetes.

	Overall Population			With Diabetes		Without Diabetes	
Goal	Eze/Statin vs. Statin	OR[a] (95% CI)	OR[b] (95% CI)	Eze/Statin vs. Statin	OR[c] (95% CI)	Eze/Statin vs. Statin	OR[d] (95% CI)
LDL-C <2.59 mmol/L	75% vs. 52%	4.23 (3.95, 4.53)	1.41 (1.30, 1.52)	85% vs. 65%	4.90 (4.25, 5.66)	71% vs. 46%	4.04 (3.73, 4.37)
LDL-C <1.99 mmol/L	45% vs. 23%	3.06 (2.70, 4.25)	1.47 (1.36, 1.58)	59% vs. 34%	4.59 (4.05, 5.19)	38% vs. 18%	3.70 (3.40, 4.02)
LDL-C <1.81 mmol/L	33% vs. 15%	4.02 (3.72, 4.34)	1.48 (1.37, 1.60)	48% vs. 25%	4.59 (4.04, 5.22)	27% vs. 11%	3.70 (3.36, 4.07)
Non-HDL-C <3.37 mmol/L	75% vs. 53%	4.13 (3.85, 4.43)	1.30 (1.21, 1.41)	82% vs. 63%	4.48 (3.90, 5.16)	72% vs. 49%	4.01 (3.70, 4.35)
Non-HDL-C <2.59 mmol/L	41% vs. 21%	3.81 (3.55, 4.09)	1.27 (1.18, 1.36)	52% vs. 29%	4.38 (3.85, 4.98)	36% vs. 17%	3.55 (3.26, 3.88)
Apo B <0.9 g/L	45% vs. 29%	2.88 (2.69, 3.09)	1.23 (1.14, 1.32)	55% vs. 38%	3.17 (2.79, 3.59)	41% vs. 25%	2.75 (2.53, 2.99)
Apo B <0.8 g/L	20% vs. 10%	3.09 (2.85, 3.35)	1.10 (1.02, 1.20)	37% vs. 22%	3.37 (2.93, 3.87)	25% vs. 13%	2.93 (2.65, 3.23)
hs-CRP <2 mg/L*	56% vs. 52%	1.20 (1.12, 1.28)	0.84 (0.78, 0.91)	50% vs. 46%	1.20 (1.06, 1.36)	59% vs. 55%	1.19 (1.10, 1.30)
hs-CRP <1 mg/L*	31% vs. 28%	1.25 (1.16, 1.34)	0.93 (0.86, 1.10)	27% vs. 25%	1.19 (1.03, 1.37)	33% vs. 29%	1.27 (1.16, 1.39)

[a]Based on logistic model with terms for first-second line, diabetes, treatment, and baseline value(s), odds ratio and 95% CI of attaining goal on EZE/Statin versus Statin.
[b]Based on logistic model with terms for first-second line, treatment, and baseline value(s), odds ratio and 95% CI of attaining goal on EZE/Statin versus Statin.
[c]Based on logistic model with terms for first-second line, diabetes, treatment, and baseline value(s), odds ratio and 95% CI of attaining goal for diabetic versus non-diabetic patients.
*The magnitudes of differences in goal attainment rates between the two treatments were small but statistically significant.

Supported by: Merck

1296

Comparative efficacy of fenofibrate/pravastatin/ezetimibe and simvastatin/ezetimibe therapies in type 2 diabetic patients with combined hyperlipidaemia and cardiovascular disease

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Background and aims: Very high risk patients with type 2 diabetes (T2D) and cardiovascular disease often require combination therapy to achieve recommended LDL-cholesterol (LDL-C) and non HDL-cholesterol (non-HDL-C) goals. This study evaluated the efficacy and safety of Fenofibrate (F) 160 mg/Pravastatin (P) 40 mg fixed dose combination and Ezetimibe (E) 10 mg compared to Simvastatin (S) 20 mg and E10 mg in T2D patients with cardiovascular disease and not at goals on S20 mg.
Materials and methods: This randomized, double-blind, parallel group study was conducted at 73 European centers. After a 6-week run-in period on S20 mg, 273 patients with non-HDL-C ≥ 100 mg/dL or LDL-C ≥ 70 mg/dL and triglycerides (TG) 150-600 mg/dL were randomized (week 0) for a 12-week treatment period to the F160 mg/P40 mg and E10 mg triple therapy or the combination of S20 mg and E10 mg, followed by a 12-week open-label period where all patients received the triple therapy. The primary efficacy comparison was the mean percent (%) changes in non-HDL-C (F/P+E vs S+E). Secondary end-points included LDL-C, HDL-C, TG, ApoB and fibrinogen.
Results: At week 12, no significant differences were observed between the F160/P40+E10 group and the S20+E10 group in reducing non-HDL-C (-21.2% vs -24.7%; p=0.09) and ApoB (-15.7% vs -18.1%; p=0.149), and in increasing HDL-C (+3.5% vs +0.5%; p=0.066). The changes in LDL-C were -19.8% in the F160/P40+E10 group and -25.1% in the S20+E10 group (p=0.05). The triple therapy was more effective than S20+E10 in reducing TG (-22.8% vs -8.2%; p=0.007) and fibrinogen (-11.8% vs +0.6%; p<0.0001). The triple therapy was generally well tolerated with a safety profile comparable to the S20+E10 combination therapy. Especially no cases of myopathy or rhabdomyolysis were reported.
Conclusion: The Fenofibrate160 mg/Pravastatin 40 mg fixed-dose combination associated with Ezetimibe 10 mg was a new alternative to improve the global atherogenic lipid profile in T2D patients with combined hyperlipidemia in secondary prevention.
Supported by: SMB Laboratories Belgium

1297

Effect of one year treatment with insulin and oral glucose-lowering agents on lipid levels in non-obese patients with type 2 diabetes

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Background and aims: In non-obese patients with type-2 diabetes (T2DM), metformin has been suggested to improve cholesterolaemia more so than an insulin secretagogue despite similar glycaemic control. Also, the cholesterol-lowering effect of metformin has been suggested to be enhanced by concomitant statin therapy. It is unknown if these effects persist when treatments are combined with insulin therapy. We aimed to study the effect of repaglinide (an insulin secretagogue) versus metformin both in combination with insulin therapy on lipid levels in non-obese T2DM patients. Furthermore, the effect of statin use was of interest.
Materials and methods: 101 non-obese T2DM patients (BMI ≤27 kg/m^2) with HbA_{1c} ≥6.5% on oral agents were randomised to one year treatment with insulin (biphasic insulin aspart 70/30) in combination with either repaglinide 6 mg or metformin 2g (double-masked). Insulin doses were adjusted aiming for HbA_{1c}<6.5%. Those patients who did not start or stop statin therapy during follow-up were analysed (n=88).
Results: In statin users, insulin plus metformin (n=43) significantly lowered fasting total and Non-HDL cholesterol (Non-HDLC) compared with insulin plus repaglinide (n=40) (mean [95% CI] baseline-adjusted difference between treatments for Non-HDLC: −0.26 mmol/l [−0.49; −0.02], p=0.039). In patients not using statins (n=5), no statistical significant differences of plasma lipid levels was observed between patients using insulin plus metformin versus insulin plus repaglinide. Conclusions were similar after adjusting for

changes in statin doses. Insulin doses and HbA_{1c} were similar between treatments (previously reported).
Conclusion: In non-obese T2DM patients using statins one-year treatment with insulin plus metformin rather than insulin plus an insulin secretagogue might improve proatherogenic cholesterolaemia. This supports potential cardioprotective effects of metformin when combined with insulin even in statin users.
Supported by: Novo Nordisk A/S

1298

Loss of the association between plasma Pro-protein Convertase Subtilisin/Kexin type 9 (PCSK9) and LDL-apoB100 catabolism in type 2 diabetes
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Background and aims: Pro-protein convertase subtilisin/kexin type 9 (PCSK9) is an important regulator of LDL metabolism because of its ability to facilitate degradation of LDL-receptors. It has recently been shown, in non-diabetic men, that plasma PCSK9 level was inversely correlated with LDL apoB100 fractional catabolic rate (FCR) suggesting an influence of plasma PCSK9 on LDL catabolism. However, the association between plasma PCSK9 and LDL catabolism remains unknown, in patients with type 2 diabetes.
Materials and methods: This prompted us to perform a kinetic study of LDL-apoB100, using C^{13} leucine, in 38 individuals (20 men, 18 women) including 23 non diabetic normolipidemic subjects and 15 patients with type 2 diabetes.
Results: Plasma PCSK9 levels were not significantly different between non diabetic subjects and patients with type 2 diabetes (271 ± 109 vs. 301 ± 96 ng/ml). In the non diabetic group, plasma PCSK9 was positively correlated with age (r=0.38, p=0.018), LDL-cholesterol (r= 0.43, p=0.006), apoB (r=0.44, p=0.005) and inversely correlated with LDL-apoB100 FCR (r=-0.52, p=0.001). In multivariate analysis, LDL-apoB100 FCR was independently associated with PCSK9 (p=0.0006) and gender (p=0.038), but not with age or BMI. Plasma PCSK9 concentration explained, in this non diabetic population, 37% of the variance in LDL-apoB100 FCR. On the other hand, in the population with type 2 diabetes no correlation was found between plasma PCSK9, on the one hand, and LDL-cholesterol, apoB and and LDL-apoB100 FCR, on the other hand. In both groups, no correlations were found between plasma PCSK9 and LDL-apoB Production Rate.
Conclusion: Our data indicate that plasma PCSK9 influences significantly the catabolism of LDL-apoB100 in individuals without diabetes but not in patients with type 2 diabetes. The reasons for this loss of association between plasma PCSK9 and LDL catabolism, in type 2 diabetes, remain to be determined.

1299

Effects of extended release niacin/laropiprant on lipoprotein subfractions in patients with type 2 diabetes mellitus
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Background and aims: Lipid management guidelines emphasize reducing LDL cholesterol as the primary goal of pharmacologic treatment. However other lipoprotein parameters, including increased plasma concentrations of small LDL, and IDL particles, and reduced plasma concentration of medium/large HDL particles, may also place patients at increased risk for coronary heart disease. Patients with T2D are especially prone to this atherogenic lipoprotein phenotype Extended release niacin/laropiprant (ERN/LRPT) is a fixed-dose combination tablet containing 1 g of ERN and 20 mg of LRPT, a prostaglandin D_2 receptor antagonist that reduces ERN-induced flushing while preserving ERN's LDL-C and triglyceride lowering, and HDL-C raising effects. In this assessment, the effects of 12 weeks of treatment with ERN/LRPT in patients with T2D on plasma lipoprotein particles were evaluated.
Materials and methods: In a multicenter, double-blind, placebo-controlled, 36-week study, T2D patients (n=796) were randomized 4:3 to ERN/LRPT (1 tablet / day) or placebo (PBO). After 4 weeks 2 tablets / day of ERN/LRPT were given for the remainder of the study. HDL, LDL, VLDL, and chylomicron particle size and concentration were evaluated at week 12 by NMR. Lipoprotein subfraction data are reported as summary statistics without adjustment for covariates.
Results: At week 12, ERN/LRPT produced significant_(p≤0.001 for all) changes in LDL-C (-17.9%), HDL-C (23.2%), and triglycerides (-23.1%). Compared with PBO, ERN/LRPT treatment was associated with a relative shift in the plasma concentration of HDL particles from small to large diameter, and a decrease in the plasma concentrations of all LDL and IDL particles (Table). ERN/LRPT also produced large reductions in the plasma concentrations of all VLDL and chylomicron particles relative to PBO.
Conclusion: In patients with T2D, 12 weeks of treatment with ERN/LRPT shifted the overall lipoprotein profile toward a potentially less atherogenic pattern: reducing the plasma concentration of small LDL and IDL particles and increasing the plasma concentration of large HDL particles compared with PBO.

	PLASMA LIPOPROTEIN CONCENTRATIONS MEDIAN baseline / MEDIAN CHANGE from Baseline at Week 12 *						
TREATMENT	HDL Small†	HDL Medium†	HDL Large†	LDL Very Small‡	LDL Small‡	LDL Medium / Small‡	LDL Large‡	IDL‡
ERN/LRPT (N=382)	23.5 / -2.9	0.7 / 0.0	5.7 / 2.0	602.0 / -100.0	746.5 / -116.0	148.0 / -12.0	350.0 / -24.5	15.5 / -3.5
PBO (N=304)	23.9 / -1.0	0.8 / 0.0	6.0 / 0.0	634.0 / -9.0	793.0 / -7.0	163.0 / -3.0	325.0 / 1.0	20.0 / -1.0

*evaluation by NMR: initial summary statistics, full analysis set; †µmol/L; ‡nmol/L

Supported by: Merck Sharp & Dohme Corp

1300

Role of HDL glycation and glycoxidation in counteracting the inhibitory effect of oxidized LDL on endothelium-dependent vasorelaxation
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Background and aims: In healthy normolipidaemic and normoglycaemic control subjects, HDL are able to reverse the inhibition of vasodilation induced by oxidized LDL. We have previously shown that in type 1 diabetic patients, HDL do not protect against the inhibition of endothelium dependant vasorelaxation induced by oxidized LDL. This defect was not explained by abnormalities in HDL composition, size or paraoxonase activity. The aim of this study was to analyse the role of glycation or glycoxidation of HDL on this vasodilation effect.
Materials and methods: Blood samples were collected from healthy patients. Extracted HDL particles, separated by ultracentrifugation, were glycated or glycoxidized in vitro. Vasoreactivity was evaluated by the relaxation response to acetylcholine of rabbit aorta rings pre-contracted with noradrenaline, before and after two hours incubation with or without different lipoprotein fractions (Krebs'buffer; ox-LDL; normal, glycated or glycoxidized HDL alone; normal, glycated or glycoxidized HDL + ox-LDL).
Results: The lipid composition of normal, glycated and glycoxidized HDL was similar. The mean of the Fructosamine/ApoA1 ratio was 17.58 µmole /g of protein for normal HDL and 48.67 and 53.63 µmole /g of protein for glycated and glycoxidized HDL, respectively. The oxysterol level of glycoxidized HDL was significantly higher than that for normal and glycated HDL (p=0.009 and p=0.016 respectively). Oxidized LDL inhibited endothelium vasodilation (maximal relaxation (Emax) = 53.34 ± 27.06 vs 98.67 ± 10.16 % for incubation in Krebs'buffer, p<0.005). Normal HDL were able to counteract the oxidized LDL-induced inhibition of vasorelaxation (Emax = 78.75 ± 19.72 vs 53.34 ± 27.06 %, p=0.003), whereas glycated and glycoxidized HDL had no effect (p=0.35 and 0.092 respectively). No difference was observed between glycated and glycoxidized HDL (p=0.28).
Conclusion: Our data indicate that glycation of HDL, is responsible for the inability of HDL particles to counteract the oxidized LDL-induced inhibition of endothelium-dependent vasorelaxation. The oxidation of glycated HDL brings no additional effects. Glycation of HDL is probably one important factor that explains the absence of the vasodilation effect of HDL particles in patients with diabetes.

1301

Insulin restores selective insulin resistance in type 2 diabetic mellitus patients with severe hypertriglyceridaemia

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Introduction: Severe hypertriglyceridaemia (SHTG) is a recognised complication of type 2 Diabetes Mellitus (T2DM) and poses significant risk of premature atherosclerosis and pancreatitis. Altered regulation of triglyceride (TG) metabolism by hepatic and adipose tissue remains critical in T2DM patients. Hyperglycaemia is associated with resistance of hepatic transcription factor *FoxO1* and adipose tissue lipoprotein lipase (LPL) to the actions of insulin, resulting in uncontrolled gluconeogenesis and reduced hydrolysis of serum TG. Conversely, recent research has highlighted that hepatic TG production remains sensitive to circulating insulin, through the activation of transcription factor *SREBP*-1c, perpetuating the detrimental effects of hyperglycaemia and SHTG. We report the novel use of continuous intravenous (IV) insulin to restore this selective insulin resistance and reduce the risk of SHTG in T2DM.
Methods: Patients with hyperglycaemia and SHTG (serum TG >15mmol/L) treated with continuous insulin were retrospectively evaluated within our centre. Demographics, admission details, lipid profiles, glycaemic control and adverse events were recorded. Patients receiving treatment dose heparin were excluded to minimise effect on LPL.
Results: Fifteen patients were reviewed. Mean patient age 46 (27 - 70) years. Patients included 8 Caucasians, 5 Afro-caribbeans and 2 Indo-asians. New onset T2DM was diagnosed in 7 cases. Mean disease duration in the remaining cohort was 60 (2 - 96) months. Median admission HbA1c measured 9.6% (6.1 - 16.1). Pre-admission Insulin was utilised in 75% (n=6) and Metformin in 25% (n=2) patients. Acute pancreatitis was diagnosed in 3 patients prior to insulin infusion. Median admission serum TG measured 26.23mmol/L (15.09 - 48.43) and serum cholesterol 11.24mmol/L (5.39 -19.62). Continuous insulin was infused for an average 48 hours (24 - 72). Median serum TG reduced to 15.79mmol/L (0.79 - 36.59) following 24 hours insulin infusion (n=15) and 12.15mmol/L (5.74 - 32.49) at 48 hours duration (n=8). Insulin infusion continued for 72 hours in 7 patients with median serum TG measuring 10.20mmol/L (5.74 - 24.03). Median discharge serum TG measured 5.75mmol/L (0.79 - 11.66) and serum cholesterol 5.90mmol/L (3.65 - 10.74) correlating with significant reduction in serum TG following IV insulin (p=<0.05). Median length of hospitalisation was 4 days (3 - 15). Concomitant lipid lowering therapy included statins (n=9) and omega-3-acid ethyl esters (n=9). Continued administration of Fenofibrate occurred in 2 patients. Prophylactic low molecular weight heparin was given to all patients (Enoxaparin 20-40mg).
Conclusion: These results detail SHTG associated with hyperglycaemia in a heterogeneous group of T2DM patients ranging from new onset diabetes to established disease. Yet, in all patients, administration of continuous insulin appears to not only achieve normoglycaemia but also dramatically correct SHTG. Insulin stimulates the action of LPL in adipocytes and these findings support this theory owing to the rapid clearance of serum TG. In addition, we speculate that administration of insulin may also regulate gluconeogenesis and hepatic TG synthesis in hyperinsulinaemic patients; restoring selective insulin resistance. Ultimately, the administration of continuous insulin in T2DM patients with SHTG is a simple method of reducing the immediate risk associated with this metabolic complication.

1302

Underutilisation of statins in patients with type 2 diabetes treated with an antihyperglycaemic regimen

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Background and aims: Patients with type 2 diabetes (T2DM) are at a high risk for cardiovascular (CV) events. Diabetic dyslipidaemia is a key CV risk factor and statin therapy has been demonstrated to reduce CV risk. Therefore use of statins is widely recommended in current treatment guidelines for patients with T2DM. The purpose of this study was to estimate the proportions of patients with T2DM treated with an antihyperglycaemic agent (AHA) who needed a statin therapy based on ADA recommendations and of patients who received statin therapy in clinical practice.
Materials and methods: The study used the GE Healthcare's electronic medical record database, and included patients who were ≥25 years with T2DM and received AHA (oral or insulin) prescription (Rx) between 7/2006 and 6/2008 (index period). The index date was the date of the first AHA Rx within the index period. Patient eligibility for statin therapy according to the ADA Standards of Medical Care in Diabetes (2008) was assessed using patient medical records 1 year prior to (baseline) and 1 year after (follow-up) the index date. Concomitant statin use with AHA therapy was based on Rx records during the follow-up period. Logistic regression was performed to estimate the likelihood of statin use in relation to baseline characteristics, co-morbidities, clinical and laboratory measures, and medication use.
Results: Of the 113,906 patients with T2DM treated with AHAs, 48% were male and mean (SD) age was 63 (13) years. At baseline, LDL-C was ≥ 2.6 mmol/L (100 mg/dL) in 49% of the patients not on a lipid-lowering agent (LLA) and in 34% of the patients on a LLA. While 98% of the patients met ADA eligibility standards for statin therapy, only 64% of patients actually received a statin Rx during the follow-up period. The adjusted logistic regression showed that older age, male, smoking, baseline antihypertensive Rx, and baseline blood thinner Rx are factors associated with increased likelihood of statin use (all p<0.001).
Conclusion: Although nearly all patients with T2DM on AHA were eligible for statin therapy per ADA recommendations, only 64% were treated with statin in our study. This indicates that statins are underused in current clinical practice and a more integrated approach is needed to improve statin utilization to reduce CV risk in patients with T2DM.
Supported by: Merck

PS 130 Endothelial function

1303

Arterial stiffness and endothelial dysfunction in type 1 diabetes mellitus

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Background and aims: Subjects with type 1 diabetes mellitus (T1DM) have a very high cardiovascular (CV) risk, which is not fully understood by the classical CV risk factors. Arterial stiffness (AS) could provide some additional information regarding CV risk in these subjects. Endothelial dysfunction is involved in the atherosclerotic process. The studies regarding the relationship between AS and endothelial dysfunction in T1DM are few and contradictory. We aimed at evaluating the relationship between: (1) AS and CV risk factors and (2) AS and endothelial dysfunction.
Materials and methods: Sixty-eight subjects with T1DM were evaluated by (1) sex, age, BMI, WHR, systolic (SBP) and diastolic (DBP) blood pressure, smoking, HbA1c and lipid profile; (2) microvascular complications; (3) insulin resistance (by way of the William's mathematical estimation of the glucose disposal rate -eGDR-); (4) AS assessed as aortic pulse wave velocity (PWV) measured by applanation tonometry (SphygmoCor®) and (5) endothelial dysfunction assessed non-invasively by reactive hyperemia-peripheral arterial tonometry (RH-PAT; EndoPAT2000).
Results: We evaluated 34 men (aged 35.5±9 years, diabetes duration: 14.5±8.5 years, BMI: 26.0±3.3 kg/m2, WHR: 0.91±0.08, SBP: 131.5±10.9 mmHg, DBP: 76.7±7.1 mmHg, smokers: 36.4%, fasting plasma glucose (FPG) 156.2±68.7 mg/dl, HbA1c: 7.4±1.1%, LDL: 100.5±26.1 mg/dl, microvascular complications: 27.3%) and 34 women (aged 35.2±11.2 years, diabetes duration: 12.9±8 years, BMI: 25.3±3.9 kg/m2, WHR: 0.81±0.07, SBP: 118.3±9.6 mmHg, DBP: 69.1±7.9 mmHg, smokers: 35.3%, FPG 172.7±64.0, HbA1c: 7.97±1.2%, LDL 104.5±27.5mg/dl, microvascular complications: 23.5%). In the whole group, PWV correlated positively with age (r=0.53, p<0.001), diabetes duration (r=0.27, p=0.028), BMI (r=0.57, p<0.001), WHR (r=0.38, p=0.001), SBP (0.36, p=0.003) and DBP (r=0.26, p=0.031). In addition, we found a negative correlation between PWV and eGDR (r=-0.31, p=0.011). No association was found between PWV and smoking, lipid profile, FPG, HbA1c, microvascular complications and RH-PAT. In multivariate regression analysis, age (ß=0.46, p<0.001) and BMI (ß=0.40, p<0.001) were the only predictors of PWV (model R=0.686). Although PWV was similar in both genders (men: 7.13±1.34 vs women: 6.86±1.71, p=0.48), in multivariate regression analysis stratifying for sex, age (ß=0.43, p=0.007) and BMI (ß=0.34, p=0.029) were the only predictors of PWV in men (R=0.609) and age (ß=0.29, p=0.020), BMI (ß=0.48, p<0.001) and diabetes duration (ß=0.35, p=0.012) in women (R=0.786).
Conclusion: In subjects with T1DM, the main determinants of PWV were age and BMI. Additionally, diabetes duration was another determinant in women. We did not find any other independent relationship between PWV and the rest of the classical CV risk factors or glycemic control, suggesting that the measurement of PWV could be useful in the assessing of CV risk. Finally, we did not find any association between PWV and endothelial dysfunction measured by RH-PAT.
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1304

Endothelial dysfunction and arterial stiffness are linked in hypertensive patients with type 2 diabetes mellitus

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Background and aims: In diabetic as well as in hypertensive patients, increased arterial stiffness and endothelial dysfunction both have been both associated with an increased risk of cardiovascular events. Arterial stiffness has been usually ascribed to vascular structural alterations, although a "functional" component contributing to the compliance of large arteries has been demonstrated recently. An inverse correlation between endothelial dysfunction and arterial stiffness was reported in healthy subjects, while this interrelationship has been poorly explored in subjects at high risk of cardiovascular disease, such as patients with hypertension or diabetes. In this study this relationship has been evaluated in hypertensive patients with or without type 2 diabetes mellitus.
Materials and methods: Hypertensive patients with (DM+, n. 69) and without diabetes (DM-, n. 68), matched for age, gender, blood pressure, duration of hypertension and number and class of antihypertensive medications, were included. Brachial artery endothelium-dependent flow-mediated dilation (FMD) and endothelium-independent dilation by 25 μg sublingual glyceryl trinitrate (GTN) were assessed by high-resolution ultrasound and computerized edge detection system. Applanation tonometry was used to measure aortic pulse wave velocity (aPWV), as index of arterial stiffness.
Results: DM+ patients showed higher BMI, waist circumference, blood glucose and HbA1c values, as well lower total, LDL and HDL cholesterol levels compared with DM-. Urinary albumin to creatinine ratio (UACR) was within the normal range in both groups and no difference in hsCRP was found. DM+ showed lower FMD (3.3±2.0 vs 5.0±3.2%, p<0.0001) than DM-, while GTN response was similar. Aortic PWV was higher in DM+ than in DM- patients (10.3±12.2 vs 8.8±1.4 m/s, p<0.0001). The difference remained statistically different (p=0.003) when mean BP, age and BMI were considered as covariates. An increased PWV, defined on the basis of the cut-off of 8.3 m/s, was found in 84% of DM+ and in 64% of DM- (p=0.006). Multiple regression analysis, driven by simple regression, was used to identify independent predictors of aPWV, including in the model age, systolic blood pressure, heart rate, blood glucose, HDL cholesterol, tryglicerides, BMI and waist circumference. Age (r2=0.05, p=0.002), systolic blood pressure (r2=0.08, p=0.001), BMI (r2=0.07, p=0.003), and FMD (r2=0.04, p=0.03), were independently related to PWV (full model r2=0.45). In DM+, FMD (r2=0.11, p=0.003), systolic BP (r2=0.14, p=0.006) and BMI (r2=0.08, p=0.02), remained independent predictors of aPWV (full model: r2=0.33). In DM-, age, but not FMD, was an independent predictor of PWV (r2=0.16).
Conclusion: We demonstrated that increased aortic stiffness is associated with endothelial dysfunction in hypertensive type 2 diabetic patients. The absence of this correlation in normoglycemic hypertensive patients, who have lower aortic stiffness and better endothelial function, suggests specific mechanisms related to the presence of diabetes.

1305

Circulating omentin-1 is associated with endothelial function independently of insulin sensitivity in subjects with altered glucose tolerance

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Background and aims: Omentin-1 is a novel soluble lectin expressed exclusively in the endothelial cells of the blood vessels found in the visceral adipose tissue. Omentin has a vasodilating effect on isolated blood vessels, which is mediated through endothelium-derived NO. To gain insight in the relationship between obesity and cardiovascular risk factors, we aimed to explore the interaction among circulating omentin-1, metabolic parameters and endothelial function according to glucose tolerance status in a human cross-sectional study.
Materials and methods: Circulating omentin-1 (ELISA) was studied in 155 healthy Caucasian men according to glucose tolerance status. Insulin sensitivity was measured using the frequently sampled intravenous glucose tolerance test in 106 subjects. Vascular reactivity was measured by high-resolution ultrasound of the brachial artery in 58 of these subjects.
Results: Circulating omentin-1 concentration was significantly increased in non-obese compared with obese subjects with altered glucose tolerance (AGT, 47.6 ± 15.8 vs. 40.5 ± 13.2 ng/ml, p=0.04). In AGT subjects, serum omentin-1 was negatively associated with obesity parameters [body mass index (r=-0.24, p=0.04), waist to hip ratio (r= -0.25, p= 0.04) and fat mass (r=-0.29, 0.01)], blood pressure [systolic and diastolic blood pressure (r=-0.27, p=0.02 and r=-0.26, p=0.025, respectively)] and circulating IL-6 (r=-0.43, p=0.001) and positively linked to insulin sensitivity (r=0.36, p=0.03), endothelium-independent (r=0.50, p=0.007) and dependent (r= 0.33, p= 0.04) vasodilation. Circulating omentin-1 (p=0.02) and systolic blood pressure (p=0.01) contributed independently to endothelium-dependent but not to endothelium-independent vasodilation variance after controlling for confounding factors in AGT subjects.

Conclusion: Omentin-1 might constitute a biomarker for endothelial function in AGT subjects.
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1306

The extent of endothelial dysfunction in polycystic ovarian syndrome is moderated by obesity status
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Background and aims: Females with PCOS are at increased risk of cardiovascular disease (CVD). Recent reports suggest that endothelial dysfunction, an early marker of CVD, measured using the flow mediated dilatation (FMD) is evident in PCOS patients. Nevertheless, the supporting evidence remains equivocal, potentially due to differences in obesity and/or insulin resistance which are associated with increased CVD risk and also manifest in PCOS. The aim of this study was to examine the degree to which obesity moderates endothelial function in PCOS patients and controls using a formal meta-analytical approach.
Materials and methods: A systematic review of published studies comparing endothelial function in PCOS patients to control individuals was performed. Nine published and 1 recent unpublished study (PCOS *n*=621; control *n*=297 participants) that measured endothelial-dependent FMD were included. At whole study level PCOS patient demographics included age range of 22.7-35.2 yrs and BMI 23.8-33.8kg/m^2 while control participants age ranged between 21.9-36.7yrs and the BMI from 22.8-37.3 kg/m^2. All participants were normotensive and PCOS patients demonstrated at least two out of the three Rotterdam criteria. The FMD values for PCOS and controls were compared and meta-regressed against BMI. Data are described as mean±SD.
Results: The meta-analysed pooled reduction in FMD was found to be 3.7% (95% CI = 2.7 to 4.8%) in PCOS patients when compared with matched controls (P<0.0005). Significant inter-study heterogeneity was detected (I-square = 68%, P=0.001). Therefore, meta-regression methods were used to explore the impact of BMI status on FMD reduction. The difference in FMD between PCOS and controls was less pronounced (group difference reduced by 0.2% per kg/m2) when participants were obese (P<0.0005). There was no evident publication bias (P=0.52).
Conclusion: There is overwhelming evidence that FMD is lower in PCOS patients compared with controls matched for BMI. Nevertheless, it is apparent from this analysis that the endothelial dysfunction in PCOS is influenced by obesity; with larger differences in FMD between PCOS and control individuals when these individuals are normal weight, suggesting that obesity outweighs the PCOS effect on FMD.

1307

Integrated endothelial function assessment in morbidly obese subjects
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Background and aims: Endothelial function, particularly endothelium-dependent vasodilation is impaired in overweight subjects and is thought to contribute to their increased risk of cardiovascular events. The mechanisms responsible for the adiposity-related reduction in endothelial vasodilatation function are not completely understood. We investigated endothelial function, inflammatory and glucose metabolism parameters in morbidly obese (BMI>40kg/m2) but otherwise healthy subjects.
Materials and methods: We studied 17 (5 men, 12 women, mean age 37,1+/-8,4, BMI 44,14+/-3,9 kg/m2) normotensive obese subjects without prior diabetes diagnosis. 13 age-matched healthy non-overweight subjects served as controls. All subjects underwent oral 75 g glucose tolerance test (OGTT) with plasma insulin measurements. Fasting plasma subclinical inflammation markers (interleukin-6, IL-6; tumor necrosis factor-α, TNF- α), leptin, endothelial dysfunction parameters (intercellular adhesion molecule, ICAM; vascular cell adhesion molecule, VCAM; E-selectin, thrombomodulin) were measured. In addition, endothelial function was assessed in vivo as flow-mediated dilation (FMD) and sublingual nitroglycerin response (nitrate-induced dilation, NID) in the brachial artery in all subjects. Intima-media thickness (IMT) of caroid artery was measured as well.
Results: During OGTT, as compared to the controls, obese subjects had higher glucose level at 60 min (141±38 vs 103±36 mg/dl; p<0.03) and 120 min (121±33 vs 90±28 mg/dl; p<0.03) as well as fasting insulin level (28±25 vs 10±8 μIU/mL; p<0.04) and insulin level at 60 min (115±63 vs 33±21 μIU/mL; p<0.001). Moreover, in the obese subjects HOMA-IR (6.2±5.4 vs 2.34±1.86, p<0.03), TNF-α (24.1±11.0 vs. 13.3±6.9 ng/ml), IL-6 (6.82±4.1 vs 3.85±1.4 pg/ml; p<0.001), leptin (63.2±16.3 vs 26.2±18.1 mg/ml), VCAM (857±312 vs 613±210 ng/ml; p<0.02), sE-selectin (40.7±17.6 vs 25.8±19.6 pg/ml, p<0.04), thrombomodulin (3.67±2.8 vs 1.1±0.53 pg/ml, p<0.004) were greater than in the controls, whilst NID (8.67±2.2 vs 14.5±3.2%, p<0.001) and FMD (4.73±1.9 vs 8.4±1.9%, p<0.001) parameters were lower than in the controls. IMT was higher in the study group (0.69±0.12 vs 0.59±0.1 mm, p<0.04). There was a significant and remarkable correlation between body weight and NID (r = -0.69, p<0.04) as well as FMD (r = -0.7; p<0.04) in the obese subjects, but a positive one with IMT (r=0.48; p<0.04). Glucose at 60 and 120 min of OGTT inversely correlated with NID (r = -0.51 and r= -0,54; respectively, p<0,04) and FMD (r= -0,76 and r= -0.63; p<0.04), also plasma insulin at 60 and 120 min showed the same relationship with NID (r = -0.56 and r= -0.45; respectively, p<0.04) and FMD (r= -0.76 and r= -0.51; respectively, p<0.04). There were no differences between both groups in regard to plasma lipid profile, systolic and diastolic blood pressure.
Conclusion: Non-diabetes morbidly obese subjects already present with mild post-challenge hyperglycemia and hyperinsulinemia as well as endothelial dysfunction and sucblinical inflammation. The results of our study suggest that vascular injury associated with obesity precedes diabetes development. This finding might have important implications for adopting prevention measures of cardiovascular disease in morbidly obese individuals.
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1308

Brown fat lipoatrophy with age is sufficient to induce obesity, vascular dysfunction and vascular insulin resistance
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Background and aims: Recently, it has been described that functional brown adipose tissue (BAT) is prevalent in adult humans and has a protector role against obesity in older patients. Our aim was to study glucose metabolism and vascular alterations in BAT insulin receptor knockout mice (BATIRKO), a mouse model characterized by a conditional-dependent loss of interscapular brown fat and an insulin secretion defect.
Material and methods: In the present work, we have analyzed vascular function, vascular insulin signaling and the expression of genes involved in vascular alterations in the aorta artery from 33- and 52-weeks old Control and BATIRKO mice.
Results: BATIRKO mice at 52 weeks had a significant decrease of BAT mass, as well as a significant increase of body weight, visceral WAT mass and TNF-α and leptin circulating levels. High circulating levels of these cytoadipokines are due to a significant increase of their expression by BAT and WAT. In addition, BATIRKO mice at 52 weeks showed more severe glucose intolerance and mild fasted hyperglycemia as compared with BATIRKO mice at 33 weeks. This fact is owing to an insulin secretion defect. We have also observed a significantly reduction of endothelium-dependent relaxation induced by acetylcholine in aortic rings from BATIRKO mice at 52 weeks-old as compared with at 33 weeks-old BATIRKO mice. In contrast, endothelium-independent relaxations to sodium nitroprusside were comparable in all groups. In addition to endothelial dysfunction, we observed a higher constrictor response to angiotensin II in aortic rings in 52 weeks BATIRKO mice as compared with all other groups studied. In addition, we observed a significant increase of genes markers of vascular dysfunction (ET-1 and ICAM-1) and inflammation (MCP-1, iNOS, TNF-α, TNFRs and PAI-1) in the aorta artery from 52 weeks BATIRKO mice as compared with all other groups studied. Finally, we analyzed vascular insulin resistance as vasorelaxing response to insulin in phenylephrine pre-contracted rings. A significant decrease trend of relaxing response to insulin in aortic rings was observed in 52 weeks BATIRKO mice versus all other groups studied. Concurrently, insulin signaling was dramatically impaired in aorta artery from BATIRKO mice at 52 weeks as revealed the lack of phosphorylation of AKT (Ser473) and eNOS (Ser1177) as compared with all other groups.

Conclusion: Our results demonstrate that the lack of brown fat tissue mass during aging is sufficient to induce obesity, glucose intolerance, vascular dysfunction and vascular insulin resistance without an overall insulin resistance.
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1309

Microvascular reactivity and oxidative stress after standard breakfast in patients with recently diagnosed type 2 diabetes
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Background and aims: The aim of the study was to compare skin microvascular reactivity (MVR) with oxidative stress and metabolic parameters at fasting status and postprandially in patients with recently diagnosed Type 2 diabetes.
Materials and methods: Twenty patients with Type 2 diabetes (mean age 58±6 years, HbA_{1C} 4.8±0.5%, diabetes duration 2.3±1.3 years, metformin treatment only) were included in the study. Blood samples were taken before and after 60, 120 and 180 minutes after standard breakfast. MVR was measured before and after 60 and 180 minutes. Standard breakfast consisted of one roll (40 g), jam (20 g), butter (10 g) and 200 ml of defined supplemental nutrition Resource (in total 2006 kJ, proteins 22.36 g, carbohydrates 62.94 g, fat 15.51 g). Skin MVR was measured by the laser Doppler flowmetry during post-occlusive (PORH) and thermal hyperemia (TH). Glycemia, insulinemia and β-hydroxybutyrate (BHB) concentration were evaluated and malonyldialdehyde (MDA) and conjugated dienes (CD) were used for the estimation of oxidative stress.
Results: Blood glucose increased from baseline 6.9±0.6 mmol/l up to 8.0±1.6 mmol/l after 60 minutes and 7.6±1.2 mmol/l ($p<0.01$) after 120 minutes in the postprandial phase. Glycemia consequently decreased after 120 minutes down to baseline level (6.2 ± 0.8 mmol/l). Insulinemia increased significantly (baseline, 60, 120, 180 minutes, respectively: 39±16 - 142±82 - 106±63 - 55±39 mIU/l, $p<0.01$) while BHB decreased (0.24±0.16 - 0.16±0.06 - 0.15±0.07 - 0.16±0.06 mmol/l, $p<0.01$). MDA concentration was significantly lower after 120 minutes than at baseline (3.02±0.48 vs. 2.80±0.40 µmol/l, $p<0.05$). Changes of several parameters of MVR were detected: maximal perfusion during PORH decreased after 180 minutes compared to baseline (235±66 vs. 198±53 PU, $p<0.01$), although maximal perfusion (expressed in % of baseline perfusion) during PORH increased after 60 and 180 minutes (baseline, 60, 120 minutes, respectively: 165±46 - 237±211 - 273±194 %, $p<0.05$). Significant decrease was found in maximal perfusion during TH after 180 minutes compared to baseline (113±53 vs. 145±71 PU, $p<0.05$). Negative correlation was found between fasting glycemia and maximal perfusion during PORH ($r=-0.51$, $p<0.05$) and positive correlation was observed between the concentration of CD and velocity of perfusion increase during TH ($r=0.63$, $p<0.01$). Positive correlation was found also between fasting concentration of CD and MDA ($r=0.53$, $p<0.05$) as well as between maximal perfusion during PORH and insulinemia after 60 minutes ($r=0.65$, $p<0.01$). Statistically significant positive relation was found between blood glucose and insulin after 180 minutes ($r=0.90$, $p<0.001$) as well as between CD concentration and time to maximal perfusion during TH at the same time ($r=0.59$, $p<0.01$).
Conclusion: Significant metabolic changes were observed postprandially in patient with early stage of Type 2 diabetes in this study. In accordance with other studies, microvascular reactivity was probably mostly influenced by increased insulinemia and vasodilatory effect of insulin. Moreover, MVR may also be modulated by the oxidative stress. The relationship between MVR and insulinemia may imply that the B-cell dysfunction can consequently lead to microvascular dysfunction through the effect of insulin. However, to confirm this hypothesis, further research in this field is necessary.

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Reduced number of early circulating vascular progenitor cells and increased central arterial stiffness in polycystic ovary syndrome
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Background and aims: Subjects with Polycystic ovarian syndrome (PCOS) are at risk of type 2 diabetes and associated cardiovascular disease. The mechanism of this enhanced risk is unclear. The number and function of circulating vascular progenitor cells (VPC) and arterial stiffness are independent predictors of cardio-metabolic risk. Aim: To study the number and function of VPC and arterial stiffness in non-obese PCOS subjects as compared to age and body mass index (BMI) matched healthy controls.
Materials and methods: Subjects with a confirmed diagnosis of PCOS with BMI<30 (n=17) attending a University hospital outpatient clinic and healthy controls (n=12) were studied. VPC number was measured by fluorescence activated cell sorting. VPC function was assesed in vitro by tube formation and VPC migration assay. Augmentation index (AIx) a measure of central arterial stiffness and central aortic blood pressures were measured by applanation tonometry at the radial artery.
Results: There was no statistically significant differences between the PCOS vs. control group in, mean ± SEM, age (26.4±1.0vs. 23.2±1.5 yrs), weight (61.0± 1.2 vs. 65.9±3.1 kg), BMI (24.2± 0.8 vs. 23.0± 0.7 kg/m^2 $p=0.26$) and waist circumference (86.3± 2.5 vs. 82.1± 1.8 cm) $p>0.05$ for all. Brachial systolic blood pressure, mean arterial pressure and pulse pressures were similar between the two groups. Compared to controls subjects with PCOS had higher central SBP (103.7±2.4 vs. 94.9± 2.2 mmHg $p=0.01$), central DBP (75.6±1.8 vs. 69.7±2.4 mmHg $p=0.06$) and central pulse pressure (28.2±1.0 vs. 25.1±1.1 $p=0.04$). AIx was significantly, more than 3 fold, higher in PCOS subjects compared to control (18.4±1.9 vs. 4.9± 2.0 $p<0.0001$). Subjects with PCOS had reduced a significantly reduced number of the early VPC CD34+133+ , mean ±SEM, 328.1± 47.9 vs. 591.0±120.5 $p=0.02$. Other putative VPC (CD34+KDR+, CD34+133+KDR+) were not statisically different between groups. VPC function was not impaired in PCOS as compared to healthy controls. CD34+133+ VPC number and AIx was not correlated.
Conclusion: Non obese PCOS is characterized by reduced numbers of early VPC but preserved function. PCOS subjects have increased central arterial stiffness. These two unrelated changes may explain the enhanced cardio-metabolic risk of this population.

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Hypertension and neuropathy are the main determinants of impaired total arterial compliance in type 2 diabetic patients
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Background and aims: Total arterial compliance (TAC) of the systemic arterial tree may be estimated by left ventricle stroke volume index/ pulse pressure ratio (i.e. a substitute for volume changes of the arterial system/ an index of arterial stiffness). This ratio has been validated against invasive measurements of arterial compliance. A low TAC level has been associated with an increased risk of subsequent cardiovascular events in hypertensive patients and in elderly men. TAC has not yet been evaluated in the diabetic population, in particular as regards to cardiac ischemic disease. Objective was to examine the determinants of TAC in asymptomatic high-risk diabetic patients with known cardiac ischemic status.
Materials and methods: We included 287 asymptomatic patients, 166 men, 59±8 years, with diabetes duration 13±7 years, with at least one additional risk factor (hypertension 73%, dyslipidemia 70%, smokers 22%, nephropathy 38%) and without heart failure. All of them were prospectively screened for silent myocardial ischemia (SMI), defined as an abnormal stress myocardial scintigraphy. TAC was calculated using echocardiographic left ventricle measurements and brachial blood pressure measurement. Cardiac autonomic neuropathy (CAN) was assessed using standard tests (deep-breathing, lying-to-standing and Valsalva).
Results: Mean TAC was 0.68±0.23 ml/m^2/mmHg. Lower TAC levels were associated with higher age ($p=0.006$), body mass index ($p=0.03$), diabetes duration ($p=0.04$) and LDL cholesterol ($p=0.004$), and with peripheral neuropathy ($p=0.001$) and hypertension ($p<0.001$), respectively), but neither with SMI nor CAN. In multivariate analysis including all the significant correlates, hypertension (OR 2.5 [1.2-5.0], $p<0.01$) and peripheral neuropathy (OR 2.2 [1.3-3.7], $p=0.004$) were independent predictors of TAC <0.56 (first tertile). In addition left ventricle stroke volume index was lower ($p=0.02$) and pulse pressure higher ($p<0.01$) in patients with than in patients free of peripheral neuropathy.
Conclusion: In high-risk but asymptomatic type 2 diabetic patients, hypertension and peripheral neuropathy are the main determinants of a reduced TAC. Peripheral neuropathy might contribute to impair TAC by altering vessel tone and vasomotion and increasing blood volume.

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Involvement of p66Shc in TNF-alpha-induced endothelial dysfunction

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Background and aims: The pro-inflammatory cytokine TNF-alpha impairs endothelial function by modulating gene expression and increasing intracellular reactive oxygen species (ROS). The p66Shc isoform has been proposed as a sensor of cellular oxidative stress, through its phosphorylation on Ser36, and mediates stress signals in multiple cell types. The aim of our study was to investigate the role of p66Shc in TNF-alpha action in human umbilical vein endothelial cells (HUVEC).
Materials and methods: Expression and phosphorylation levels of specific signaling molecules were assessed by immunoblotting techniques. Intracellular ROS generation, in the presence of the DHE probe, was evaluated by fluorimetric analysis. Gene expression was evaluated by quantitative RT-PCR (qRT-PCR). Wild-type p66Shc and mutant p66Shc, in which Ser36 had been replaced by Ala (p66Shc-Ala36), were selectively overexpressed following infection with recombinant adenoviruses.
Results: Exposure of HUVEC to TNF-alpha (10-50 ng/ml) resulted in increased E-Selectin and IL-8 mRNA levels ($p<0.05$), evaluated by qRT-PCR, and in increased intracellular ROS concentrations ($p<0.05$), assessed by fluorimetry. Treatment with TNF-alpha was also associated with increased phosphorylation of the stress kinase JNK-1/2 ($p<0.05$ at 30 min), of ERK 1/2 ($p<0.05$ at 30 min), and of p66Shc on Ser36 ($p<0.05$ at 30 min). Pre-incubation of HUVEC with the JNK inhibitor SP600125 prevented JNK activation by TNF-alpha and the effect of this cytokine on E-Selectin mRNA ($p<0.05$ vs TNF-alpha alone), but not that on IL-8 gene expression, and also reverted TNF-alpha-induced p66Shc phosphorylation on Ser36 and ROS generation. By contrast, treatment of HUVEC with the ERK inhibitor PD98059 blocked TNF-alpha-induced ROS production ($p<0.05$ vs TNF-alpha alone), but had no effects on E-Selectin gene expression and p66Shc phosphorylation on Ser36. We next obtained a selective 3-fold overexpression of p66Shc in HUVEC by adenoviral transfer (HUVEC/p66Shc). HUVEC/p66Shc showed increased p66Shc Ser36 phosphorylation both under basal conditions and following exposure to TNF-alpha ($p<0.05$ vs controls). This was associated with an increase in E-Selectin mRNA and ROS levels, both basally and after TNF-alpha exposure ($p<0.05$ vs controls). Pretreatment of HUVEC/p66Shc with the JNK inhibitor SP600125 significantly reduced the induction of E-Selectin mRNA levels and ROS synthesis following TNF-alpha ($p<0.05$). In addition, pretreatment of HUVEC/p66Shc with the ERK inhibitor PD98059 prevented TNF-alpha-induced ROS generation ($p<0.05$), but did not modify p66Shc phosphorylation on Ser36. Conversely, overexpression of a phosphorylation-defective p66Shc protein, in which Ser36 had been mutated to Ala, did not augment E-Selectin mRNA and ROS levels beyond those found in wild-type cells.
Conclusion: p66Shc acts as a novel signaling intermediate in the TNF-alpha-MAPK pathways mediating endothelial cell dysfunction, and its action requires p66Shc phosphorylation at Ser36.
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Liraglutide down regulates endoplasmic reticulum stress in human endothelial cells exposed to hyperglycaemia

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Background: The endoplasmic reticulum (ER) is a key organelle where membrane and secreted proteins fold into their tertiary and quaternary structure. The ER stress occurs when there is an accumulation of unfolded/misfolded proteins due to disruption of ER homeostasis. The accumulation of unfolded proteins results in the activation of the unfolded protein response (UPR) which is regulated by proteins such as IRE1α, PERK and ATF6. ER stress increases PERK activity, which phosphorylates eIF2α to reduce protein translation. ER contains molecular chaperone proteins including PDI, calnexin, Ero1-Lα and Grp78/BiP and others that promote oxidative protein folding. Data on pancreatic beta cells function indicate that augmented ER stress together with reduced insulin signalling both occur before the onset of frank diabetes. It has also been observed that ER stress may play a causative role in diabetic atherogenesis. Also recent data in mice suggests that hyperglycaemia increases intracellular ER stress, prior to the onset of atherosclerosis. Liraglutide is a GLP-1 analogue that has been proven to enhance insulin signalling and reduce apoptosis in pancreatic beta cells. Investigating its effectiveness in reducing ER stress levels in endothelium might be of great use to determine its capacity in ameliorating not only the pancreatic function and insulin sensitivity, but also prevent atherogenesis and thus cardiovascular complications in diabetics.
Materials and methods: Confluent human vascular endothelial cells (HUVECs) were exposed to a 15mM high glucose media with (HGL) or without (HG) 100nM of liraglutide. Controls were kept in a 5mM normal glucose media with (NGL) or without (NG) 100nM liraglutide with 10mM of mannitol for osmotic balance. After 12 hours of exposure to the hyperglycaemic media, proteins from all the conditions were extracted. Protein analysis was conducted by western blotting.
Results: HuVEC cells exposed to hg media lead to a significant ($*p<0.01$) up regulation of all the ER stress markers as detailed: PDI, calnexin, BiP, Ero1-Lα, IRE1, phospho-eIF2α, compared to cells treated with ng. However in cells exposed to hg and liraglutide there was a significant reduction in ER stress protein expression levels compared to hg alone (PDI, BiP, Ero1-Lα, IRE1, phospho-eIF2α: $p<0.01$; calnexin, $p<0.05$) in HUVEC cells treated with and high glucose. Liraglutide had no additional effects on the basal expression of ER stress markers in normal glucose compared to control.
Conclusion: Our *in vitro* data demonstrates that liraglutide significantly down-regulates ER stress markers in endothelial cells exposed to high glucose levels. This study indicates that liraglutide has additional beneficial effects to reduce ER stress and may support prevention of atherogenesis and thus cardiovascular complications in diabetic patients.
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Tissue kallikrein is essential for invasive capacity of circulating proangiogenic cells

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Background and aims: The function of circulating proangiogenic cells (PACs) is altered in diabetes and inversely correlated with severity of vascular complications, but underpinning mechanisms remain unclear. We investigated the possibility that components of the kallikrein-kinin system, which are constitutively expressed in human PACs, may be downregulated in diabetes thus contributing to reduced PAC motility and invasiveness.
Materials and methods: Type 2 diabetic patients (T2D, n=22) and age- and gender-matched healthy subjects (n=16) were studied. Circulating mononuclear cells (MNCs) and culture-selected PACs were analyzed for expression of human tissue kallikrein (hK1) and kinin B2 receptor (B_2R) by Q-RT-PCR, western blotting, immunofluorescence staining, and flow cytometry. Secretion of hK1 by PACs was determined by measuring hK1 levels in conditioned media by ELISA and enzymatic activity assay. Moreover, the functional role of the hK1/B_2R duo was assayed by analyzing *in vitro* cellular invasive potential (modified Boyden chambers assay), proangiogenic action (matrigel assay) and activation of matrix metalloproteinase-2 (MMP2, in situ- and gelzymography) in the presence or absence of hK1 silencing by siRNA, B_2R antagonism by icatibant or MMP inhibition by GM6001. The activity of Akt was assayed as a measure of B_2R coupling to its downstream signaling machinery. Adenovirus-mediated gene transfer of hK1 (*Ad.hK1*) and B_2R (*Ad.B_2R*) was used to rescue the impaired T2D PACs phenotype.
Results: The two groups did not differ in smoking habit and LDL levels. T2D patients had higher BMI (30.2 ± 6.4 vs. 25.2 ± 3.5% in healthy) and Hba1c levels ranging between 5 and 7.5 (average value 6.6 ± 0.8), all patients were on diabetic diet, 18 out 22 were on metformin therapy. MNCs and culture-selected PACs from healthy subjects express and release mature hK1 protein. HK1 gene silencing impaired migration/invasion and proangiogenic capacities and reduced MMP2 activity in healthy PACs. T2D PACs showed reduced invasion and proangiogenic potential (-1.8 and 2.0 fold vs. controls, $p<0.05$) and lower hK1 protein (-56.6 %, $p<0.05$), but normal hK1 mRNA abundance, pointing at a post-transcriptional defect. Furthermore, T2D-PACs expressed normal levels of B_2R, but the receptor was not conductive as verified by re-

duced Akt activity following kinin stimulation. *Ad.hK1* increased the migratory (1.6 fold) and invasive (1.8 fold) activity of healthy PACs, and stimulated their capacity to promote angiogenesis on matrigel (1.4 fold) ($p<0.05$ vs. *Ad.Null*, for all comparisons). Inhibitory experiments ascribed the effects of hK1 overexpression to B_2R- and MMP-dependent mechanisms. Importantly, *Ad.hK1* was unable to rescue the impaired invasion capacity of T2D-PACs and combined genetic engineering with *hK1* and B_2R was necessary to correct the diabetic phenotype.
Conclusion: Human PACs bear active hK1, which cooperates with B_2R and MMP2 for invasion. T2D-induced deficit of hK1 results in PACs dysfunction. In diabetes, different components of the kallikrein-kinin system need to be overexpressed to restore proper invasiveness. This study unravels new molecular mechanisms underpinning PACs dysfunction in T2D.
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Exendin-4, GLP-1(7-36) and GLP-1(9-36) stimulate proliferation of human coronary artery endothelial cells through PKA- and PI3K/Akt/ eNOS-dependent pathways
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Background and aims: We recently showed that the glucagon-like peptide-1 (GLP-1) receptor is expressed in human coronary artery endothelial cells (HCAECs) and that GLP-1 improves endothelial dysfunction in type 2 diabetic patients with coronary artery disease. Exendin-4 is a stable GLP-1 receptor agonist and has been approved for clinical use against type 2 diabetes. In contrast to their glycemic effects, the role of GLP-1 and its analogues on endothelial regeneration is not known. The aim of the present study was to investigate the effects of exendin-4, GLP-1(7-36) and GLP-1(9-36) on cell proliferation in HCAECs *in vitro.*
Materials and methods: HCAECs were treated with exendin-4 (1-10 nM), GLP-1 (7-36) (100 nM) or the major GLP-1 metabolite GLP-1 (9-36) (100 nM), respectively, in serum-deficient medium in the presence of 5 mM glucose for 48 h. Phosphorylation and expression of the endothelial nitric oxide synthase (eNOS), Akt and MAP kinase were examined by Western blotting using anti-phospho-eNOS (Ser1177), anti-eNOS, anti-phospho-Akt (Ser 473), anti-Akt, and anti-phospho-MAPK ½ antibodies, respectively. ^{3}H-thymidine incorporation was assayed in 96-well plates as a measure of DNA synthesis after 48 h incubation.
Results: Incubation of HCAECs with exendin-4 for 48 h resulted in a dose-dependent increase in DNA synthesis; subsequent neogenesis was confirmed by an increased cell number. Exendin-4 dose-dependently enhanced phosphorylation of eNOS after a 48 h-incubation. The exendin-4-induced activation of eNOS was associated with an increased NO production. In addition, incubation of HCAECs with exendin-4 resulted in an increased Akt phosphorylation, The exendin-4-stimulated activation of eNOS and Akt was prevented after treatment of the cells with Rp-cAMP[S], LY294002, Akt inhibitor IV or L-NAME, the specific inhibitor for PKA, the phosphoinositide 3-kinase (PI3K), Akt and eNOS, respectively. Subsequently, the exendin-4-induced proliferation was abolished. Incubation of HCAECs with exendin-4 also caused activation of MAP kinase. However, suppressing MAP kinase activity did not affect exendin-4-induced cell proliferation. The effects of exendin-4 on enzyme activation and cell proliferation were mimicked by GLP-1(7-36) and GLP-1(9-36), but blocked by the GLP-1 receptor antagonist exendin (9-39). Co-incubation of exendin-4 with GLP-1(7-36) did not produce additive effects.
Conclusion: Our results indicate that exendin-4, GLP-1(7-36) and GLP-1(9-36) stimulate proliferation of HCAECs by PKA and PI3K/Akt/ eNOS dependent pathways. Proliferation of endothelial cells is involved in endothelial repair (arterial healing) and angiogenesis, a powerful mechanism to ensure blood supply to tissue at risk if a main artery is chronically occluded. These beneficial effects of exendin-4, GLP-1(7-36) and GLP-1(9-36) on human coronary artery endothelial cells may add yet another salutary non-glycemic property to incretin-based antidiabetic therapy, increasing its clinical utility in type 2 diabetic patients in whom endothelial dysfunction is a salient feature that adversely affect their survival.
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The role of osteopontin in endothelial progenitor cell function
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Background and aims: We, and others, have shown that endothelial progenitor cells (EPCs) obtained from patients with diabetes are dysfunctional. We previously identified the angiogenic protein osteopontin (OPN) as being down regulated in diabetic EPCs and furthermore we determined that OPN deficient mice do not recover as well as wild-type mice from hindlimb ischemia. Thus, we hypothesized that OPN may play a critical role in the angiogenic response mediated by EPCs. We aimed to establish the role OPN plays in EPC function and to determine if exposure to OPN could restore the function of OPN knockout EPCs in vivo.
Materials and methods: EPCs were obtained from OPN knockout mice as well as wild-type controls and cultured for 7 days followed by use in a matrigel tubule formation assay. Conditioned media from these cells was also used in the tubule formation assay as well as in a protein array. OPN knockout EPCs or knockout EPCs incubated with recombinant OPN were injected into the ischemic hindlimb of OPN knockout mice and laser Doppler blood flow analysis was performed immediately following surgery as well as at days 7 and 14.
Results: To elucidate the role of OPN in EPCs, a matirgel tubule assay was used to assess in vitro angiogenic potential. KO EPCs induced significantly less tubule formation than WT EPC ($p<.05$, n=3). However, knockout EPCs that were pre-incubated with recombinant OPN induced tubule formation at levels similar to WT and significantly higher than KO cells that were not incubated with OPN ($p<.05$, n=3). Further, conditioned media (CM) from WT cells induced tubule formation to the same levels as the EPCs themselves suggesting that secreted proteins are responsible for angiogenic effect. Interestingly, when KO EPC were pre-incubated with OPN, the CM media induced tubule formation to WT levels(n=3), even though there was no OPN directly in the media. Hence, we further hypothesized that OPN is acting on EPC to induce the secretion of angiogenic cytokines. Thus a protein-array was performed on EPC from WT, KO and KO EPCs exposed to OPN. WT EPCs expressed FGFα at a much higher level than KO cells. Further, WT cells expressed Il-6 and TGF-α whereas KO cells did not express these proteins at detectable levels. Interestingly, when KO cells are exposed to OPN these proteins are expressed at WT levels. Additionaly, we demonstrated that EPCs from humans and diabetic rabbits incubated with OPN had increased angiogenic potential in a tubule assay ($p<.05$ n=3) further suggesting that OPN plays a critical role n EPC funtion. When OPN KO EPCs were injected into the ischemic hindlimb of OPN KO mice blood flow to the limb was improved significantly at days 7 ($p<.05$ n=6) and 14 ($p<.01$).
Conclusion: Taken together, this data suggests that the decreased OPN expression in diabetic EPCs may contribute to their dysfunction. We propose that OPN increases the angiogenic potential of EPCs via an autocrine mechanism whereby OPN is secreted by the EPC and subsequently induces the expression of a variety of angiogenic proteins. Futher, we have demonstrated that incubation of OPN deficent EPCs with recombinant OPN is sufficent to restore function of the EPCs even when returned to an in vivo setting.
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Impaired ischaemia-induced angiogenesis in diabetic mice depends on glycaemic variability
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Background and aims: Glycemic variability (GV) is an adjunctive risk factor for diabetic vascular complications. However, neither a cause-effect correlation between glucose instability and vascular dysfunction, nor the molecular bases of such a dysfunction have been investigated previously. The aim of this study was to investigate the role of GV in diabetic vascular complications and to explore the molecular pathways modulated by glycemic "swings".
Materials and methods: Diabetes was first induced by streptozocin in 60 mice. Then 30 diabetic mice received basal insulin administration once daily plus two oral boluses of glucose solution (variable group, VG) and another 30 mice received basal insulin once daily plus two oral boluses of saline solution (stable group,SC) for a period of 30 days. Glycemia was measured eight times daily to detect GV. Post-ischemic neovascularization, induced by hindlimb

ischemia 30 days after diabetes onset, was studied and compared in VG, SC and untreated groups.
Results: Indices of GV were significantly different between VG and SG groups, whereas there was no significant difference in the mean glycemic values. Laser Doppler perfusion imaging revealed that the mean blood flow of control mice reached 94% of the pre-ischemic flow 28 days after hindlimb surgery. Perfusion recovery was significantly attenuated in SC mice compared to control mice 7, 14, 21 and 28 days after surgery. Interestingly, recovery was significantly impaired in VG compared to the SG 7, 14, 21 and 28 days after ischemic injury. Histological analysis revealed a higher increase in the capillary density of ischemic limbs in SG respect to the VG. Immunostaining and western blot analysis revealed that the impaired angiogenic response in VG occurred in association with reduced VEGF production and decreased eNOS and Akt phosphorylation.
Conclusion: This is the first murine model of GV. Our data indicate that GV causes a significant impairment of ischemia-induced angiogenesis in diabetes, regardless of average blood glucose levels, and that this impaired collateral vessel formation depends on an altered VEGF pathway. These findings provide new information to understand the biological and clinical effects of GV.

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Overexpression of glyoxalase-I improves vascular function in a rat model of diabetes

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Background and aims: The reactive advanced glycation endproduct (AGE) precursor methylglyoxal (MGO) and MGO-derived AGEs are associated with diabetic vascular complications. In this study glyoxalase-I (GLO-I) transgenic rats were used to explore whether overexpression of this MGO-detoxifying enzyme reduces levels of AGEs, and thereby improves cardiovascular function in a rat model of diabetes.
Materials and methods: Diabetes was induced in wild type (WT) and GLO-I overexpressing animals by a single tail vein injection with streptozotocin (STZ). After 12 and 24 weeks of diabetes, before termination, cardiac function was monitored by ultrasound and mean arterial blood pressure was measured intra-arterially, under isoflurane anaesthesia. After termination, vascular function of isolated mesenteric resistance arteries (MrA) was assessed by wire myography. Blood was drawn and multiple tissues were collected for further analysis. Circulating levels of glyoxal (GO), MGO, 3-deoxyglucosone (3-DG), and the AGEs Nε-(1-carboxymethyl)lysine, Nε-(1-carboxyethyl)lysine and hydroimidazolone, were assessed by high performance liquid chromatography with fluorescence or tandem mass spectrometry detection. Gene-expression levels of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) in MrA were measured by real time PCR.
Results: GLO-I activity was significantly elevated in multiple tissues of all transgenic rats. STZ treatment resulted in a fivefold increase of blood glucose concentrations irrespective of GLO-I overexpression. Levels of GO, MGO, 3-DG and AGEs were elevated in the diabetic WT rats ($p<0.01$). In diabetic GLO-I rats, GO and MGO were significantly decreased by 80 % ($p<0.05$), and plasma AGEs by 50% ($p<0.05$). In WT rats, STZ treatment significantly decreased mean arterial pressure (91±2 vs. 59±3 mmHg; $p<0.01$) and cardiac output (108±12 vs. 81±7 mL/min; $p<0.05$). GLO-I overexpression in diabetic rats significantly blunted the fall in mean arterial blood pressure (76±7 3 mmHg; $p<0.05$ compared with WT diabetic rats) without affecting the cardiac output response following administration of STZ (80±7 mL/min). In isolated MrA, STZ treatment significantly attenuated potassium-induced contractions and endothelium-dependent relaxations ($p<0.05$). These consequences of diabetes were significantly reduced by GLO-I overexpression ($p<0.05$). Furthermore, the increased mRNA levels of VCAM-1 and ICAM-1 in the MrA of the diabetic WT rats were normalised by GLO-I overexpression.
Conclusion: This study shows that overexpression of GLO-I detoxifies (methyl)glyoxal and thereby decreases (M)GO-derived AGEs in diabetic rats. The fact that GLO-I overexpression is associated with higher blood pressures and improved vascular function *ex vivo*, but not cardiac function, suggests that GLO-I protects arterial rather than cardiac function after STZ induced diabetes in rats.
Supported by: Dutch Diabetes Foundation

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The role of MRP8 and MRP14 in in stent restenosis in the diabetic rat

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Background and aims: The prevalence of type 2 diabetes mellitus is reaching pandemic proportions. The most common cause of death in diabetes mellitus is cardiovascular disease. Patients frequently undergo vascular interventions such as stenting, as a therapy for this disorder. While the occurrence of in stent restenosis has been reduced by the use of drug eluting stents, this is less effective in diabetes mellitus than in non-diabetic subjects. In addition, drug eluting stents may impair endothelial recovery post-stenting which may lead to an increased risk of in stent thrombosis, a condition with mortality of approximately 40%. Novel approaches to the prevention of in stent restenosis which do not impair endothelial function are required.
Materials and methods: The Zucker fatty rat has been chosen as model of type 2 diabetes for its metabolic characteristics. Stent implant was performed in fatty rats and lean controls in the left internal carotid artery: the animals were sacrificed before stenting, 3 days and 14 days post surgery. Samples from day 14 were plastic embedded for histomorphometry; unstented carotid arteries and 3 day post surgery arteries have been used for RNA isolation. Rat Aortic Endothelial Cells (RAOEC) were cultured in normal glucose (5.5mM) and high glucose (22mM) for 48hr before performing cell count, apoptosis, proliferation and migration assays.
Results: The Zucker fatty rat demonstrated exaggerated intimal hyperplasia 14 days post surgery in comparison to lean rats (30% vs 15%). Microarray analysis showed MRP8 and MRP14 mRNAs were upregulated ~8-fold in the carotid artery of the fatty rats versus lean controls. Quantitative PCR confirmed this result. RAOEC incubated in high glucose showed a reduction in the cell number (approx 30%) as a result of increased apoptosis and reduced proliferation. Migration of rat aortic endothelial cells (RAOECs) was impaired (60% reduction) when the cells are exposed to high glucose. Interestingly endothelial cells (ECs) incubated in high glucose displayed a 75-fold upregulation of MRP8 and a 5-fold upregulation of MRP14. MRP8 and MRP14 were cloned and shRNAs designed targetting them: RAOEC overexpressing MRP8 or MRP14 showed similar migration impairment to RAOECs exposed to high glucose; the migratory capacity was partially restored when the high glucose cells were treated with the shRNA against MRP8, but not against MRP14.
Conclusion: We show that MRP8 and MRP14 have an active role in endothelial cell dysfunction in diabetes. MRP8 and MRP14 are overexpressed in the arteries of diabetic rats and its expression is augmented in endothelial cells exposed to high glucose. MRP8 and MRP14 expression reduces the migration of endothelial cells. Endothelial cell proliferation and migration are fundamental to re-endothelialization of implanted stents, and thus limiting restenosis. Blocking MRP8 expression using shRNA we have been able to restore the migrational capacity of endothelial cells inhibited by high glucose incubation. MRP8 could be considered a good target to reduce in stent restenosis in diabetes.
Supported by: SFI, IRSCET, Medtronic

1320

Ablation of vascular endothelial phosphoinositide-dependent protein kinase 1 deteriorates the volume of blood flow in skeletal muscle

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Background and aims: Diabetes mellitus was well-known disorder complicated with lowered angiogenesis and ischemic status especially in heart and skeletal muscle. Regulation of abundant endothelial growth factor with phosphoinositide 3-kinease (PI3K) and phosphoinositide-dependent protein kinase 1 (PDK1)-Akt signal in vascular endothelial cells (ECs) is believed the most important signal during the step of angiogenesis, which might be interfered by hyperglycemia. To evaluate the systemic pathway of angiogenesis under PI3K signal, we generated ECs specific PDK1 knock out mice using Cre-loxP system and investigated the degree of impaired angiogenesis of skeletal muscles under the normo- and hyperglycemia status.

Materials and methods: Mice with PDK1 deficiency in ECs (VEPDK1KO) were obtained by crossing PDK1$^{lox/lox}$ mice with Tie2-Cre PDK1$^{lox/+}$ mice. To obtain diabetic VEPDK1KO, streptozotocin (STZ; 150mg/kg BW, i.p.) was intraperitonealy injected into 2-month-old male mice. After 1 month of injection, blood flow in lower leg-skeletal muscle was evaluated by the non-invasive blood flowmeter (ADVANCE co.).
Results: PDK1 protein levels in ECs were reduced by 80-90 % in VEPDK1KO. The phospholylation of Akt at Thr308 stimulated with insulin or vascular endothelial growth factor (VEGF) was reduced by 58% or 64%, respectively. In STZ-induced diabetic mice, body weight decreased and blood glucose was elevated, though which were similar between both genetics (BW: CON 33.9±1.7, KO 32.2±1.3, STZ-CON 21.5±1.3, STZ-KO 23.6±1.7 g, blood glucose: CON 83.3±7.9, KO 70.0±7.0, STZ-CON 152±15.1, STZ-KO143.4±34.7 mg/dl). In normoglycemic mice, the basal blood flow in lower leg was similar in both groups of mice (CON 4.0±0.9, KO 4.3±2.1 ml/min/100g), but the blood flow stimulated with insulin (10μU/g BW, 10min) was significantly lower in VEPDK1KO mice compared with the control (CON 5.9±1.4, KO 4.0±1.2 ml/min/100g). The phospholylation of eNOS, critical regulator of angiogenesis, at Ser-1177 of skeletal muscle was also decreased in VEPDK1KO by 40%. On the other hand, the basal blood flow readily decreased in diabetic mice (STZ-CON 2.4±0.8, STZ-KO 2.3±0.1 ml/min/100g), and insulin injection did not alter the blood flow in both control and VEPDK1KO mice (STZ-CON 2.2±0.2, STZ-KO 2.2±0.4 ml/min/100g). VEGF expression in skeletal muscles was similar in normoglycemic CON and KO (102 % of CON), but lowerd in diabetic mice (STZ-CON 71%, STZ-KO 68% of CON).
Conclusion: The present results suggest that the PI3K signal of ECs is critical for insulin-induced angiogenic phenomena in normoglycemic status. In diabetic state, however, other pathways related with complicated metabolic disorders might be comprehensively meaningful for angiogenesis in skeletal muscle.

1321

Human C-peptide decreases hyperglycaemia-induced reactive oxygen species (ROS) and activation of apoptotic pathways in human aortic endothelial cell

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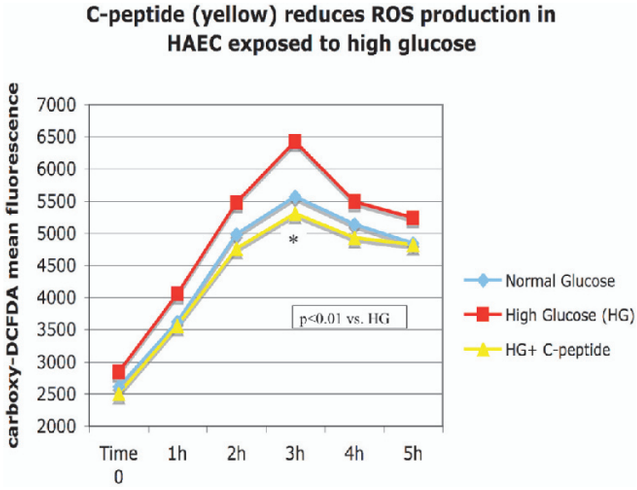
Background and aim: High glucose is toxic to endothelial cells through stimulation of reactive oxygen species (ROS) and inflammation, which cause cellular stress leading to apoptosis. Our previous studies have shown that C-peptide antagonizes high glucose-induced endothelial dysfunction by displaying a beneficial anti-inflammatory activity directly on endothelial cells. The aim of this study was to investigate whether C-peptide is able to retrieve high glucose-induced vascular damage by reducing the generation of ROS in Human Aortic Endothelial Cells (HAEC). We focused on the possible effect of C-peptide on the assembly of the NAD(P)H oxidase machinery under high glucose conditions. Activation of apoptotic pathways in HAEC was also investigated.
Materials and methods: HAEC were exposed to high glucose (25mmol/L) and Tumor Necrosis Factor-α (TNF-α; 20ng/μl) for 12-48h in presence or absence of physiologic concentrations of either C-peptide (2-10 nM) or scrambled C-peptide as a control. ROS production was determined by using the fluorescent probes 5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate (carboxy-DCFDA) by Flow Cytometry. Translocation of the NAD(P)H oxidase subunits p47phox and Rac-1 from the cytosol to the plasma membrane was investigated as a prove of the activated enzymatic machinery for ROS generation. Apoptosis was assessed by determination of cytoplasmic histone-associated-DNA-fragments and Caspase-3 activity using ELISA kits. Mitochondrial and cytoplasmic protein extracts were separated and analyzed by Western blotting using anti-Bcl-2, anti-Caspase-3, anti-p47phox and anti-Rac-1 antibodies.
Results:
• High glucose-induced ROS generation is significantly reduced by C-peptide ($p<0.01$) in HAEC to levels detected in normal glucose (see figure below). C-peptide affects translocation of NAD(P)H subunits p47phox and Rac-1 from cytoplasm to plasma membrane.
• C-peptide decreases high glucose-induced apoptosis of HAEC (38% lower vs. control) compared to high glucose alone as shown by reduced DNA fragmentation and Caspase-3 enzymatic activity.
• C-peptide increases Bcl-2 expression (survival gene) in HAEC exposed to high glucose and TNF-α.
Conclusion: Our results indicate that C-peptide at physiologic concentrations reduces high glucose-induced oxidative stress and activates cell survival pathway in endothelial cells. These results strengthen the idea that supplementation of C-peptide in Type 1 diabetic patients might prevent endothelial dysfunction and high glucose-associated vascular complications.



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PS 132 Thrombosis and haemostasis

1322

Impaired glucose metabolism and type 2 diabetes are associated with hypercoagulability as determined by thrombin generation in plasma: the role of central obesity and low-grade inflammation. The Hoorn Study

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Background and aims: Individuals with type 2 diabetes (DM2) have a greater risk for cardiovascular disease (CVD). A substantial portion of the diabetes-related CVD is due to atherothrombotic events, which could, at least in part, be explained by prothrombotic alterations in these individuals. Recently, a method was developed - the Calibrated Automated Thrombogram (CAT) - to quantitatively measure thrombin generation *in vitro;* in brief, it generates a thrombin generation curve that mimics the overall plasma coagulability potential when a thrombogenic stimulus appears. Prothrombotic alterations in individuals with impaired glucose metabolism (IGM) and DM2 have not been examined before according to this method. We have therefore investigated the extent to which individuals with IGM and/or DM2 had greater levels of thrombin generation than those with normal glucose metabolism (NGM). In addition, we examined whether any such differences were independent of other cardiovascular risk factors, such as smoking, hypertension, dyslipidaemia, (micro)albuminuria, glycemic control and (central) obesity, and/or were mediated by low-grade inflammation (high-sensitivity C-reactive protein - hsCRP).
Materials and methods: We studied 747 individuals (374 women, mean age 68.5±7.1 years) from the Hoorn Study, a population-based cohort study including individuals with NGM (n=276), IGM (n=177) and DM2 (n=294). Thrombin generation in platelet-poor plasma was measured using the CAT method and two parameters were derived: the endogenous thrombin potential (ETP, i.e. area under the thrombin generation curve, which represents the total amount of active thrombin formed after activation of the coagulation cascade) and the peak height of this curve. Data were analyzed with the use of multiple linear regression analyses.
Results: After adjustments for age, sex, prior CVD and smoking status, individuals with IGM or DM2, were characterized by a higher ETP [ß=41.39 nM*min (95%CI: 6.19 to 76.59)] and peak height [ß=8.92 nM (0.12 to 17.71)] as compared with those with NGM (but did not differ from each other with regard to these parameters). These differences were attenuated to ß=2.99 nM (-7.09 to 13.06) and 23.52 nM*min (-16;66 to 63.70), respectively, and were thus no longer significant, when further adjusted for waist circumference and hsCRP. Adjustments for other risk factors did not materially change the differences between groups, however.
Conclusion: Individuals with IGM or DM2 have higher levels of thrombin generation as compared with subjects with NGM and these differences may be explained, to a great extent, by their higher levels of central adiposity and low-grade inflammation.
Supported by: a Netherlands Heart Foundation grant to I. Ferreira

1323

Body composition as determinant of thrombin generation in plasma. The Hoorn Study

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Background and aims: The association between obesity and cardiovascular disease might, at least partially, be explained by a hypercoagulable state. The extent to which body fat mass and its distribution contribute to a hypercoagulable state is unknown. We investigated the association between body composition and thrombin generation, and evaluated the potential mediating role of low-grade inflammation (i.e. high-sensitivity C-reactive protein - hsCRP) herein.
Materials and methods: We studied 588 individuals from the Hoorn Study, a population-based cohort of individuals with normal and impaired glucose metabolism and type 2 diabetes (mean age 69.7 ± 6.5 years, 300 women) in whom total and regional (i.e. trunk, arms and legs) body composition was assessed by whole body dual-energy absorptiometry. Thrombin generation was measured using the Calibrated Automated Thrombogram, a method developed recently which generates a thrombin generation curve that mimics the overall plasma coagulability when a thrombogenic stimulus appears. The area under this curve is the endogenous thrombin potential (ETP) and represents the total amount of active thrombin formed after activation of the coagulation cascade. Data were analyzed with multiple linear regression models in men and women separately. All analyses were adjusted for age, glucose metabolism status and smoking.
Results: Men and women differed with respect to total body fat % (28±7 vs. 42±7), trunk (12.7±5.5 vs. 14.7±5.5) and peripheral (i.e. arms + legs) fat (9.5±2.9 vs.14.9±4.6) and lean (24.9±3.4 vs.17.1±2.4) masses (in kg), but not with respect to ETP (1188±234 vs.1186±204 nM*min, respectively). Total body fat % was positively associated with ETP in women [standardized regression coefficient (ß)=0.20 (95%CI: 0.09 to 0.32)], but not in men [ß=-0.02 (-0.15 to 0.11)]. Detailed analyses of regional body composition in women showed that trunk [ß=0.23 (0.05 to 0.40)], but not peripheral fat mass [ß=-0.02 (-0.18; 0.15)] was associated with greater ETP, and that there was a trend towards an inverse association with peripheral lean mass [ß=-0.12 (-0.25 to 0.01)]. The strength of the positive associations between total and trunk fat and ETP in women did not materially change after further adjustments for blood pressure, dyslipidaemia or microalbuminura, but were attenuated by 35% [to ß=0.13 (0.01 to 0.26)] and 45% [to ß=0.10 (-0.04; 0.23)], respectively, when further adjusted for hsCRP.
Conclusion: Body fat mass, in particular a central pattern of fat distribution, is associated with higher levels of thrombin generation in elderly women, but not in men. This association is partially explained by adiposity-related low-grade inflammation.
Supported by: a Netherlands Heart Foundation grant to I. Ferreira

1324

Oxidative stress is involved in the inhibitory effects exerted by high glucose on platelet sensitivity to aspirin

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Background and aims: According to therapeutic guidelines, the largest majority of type 2 diabetic patients should be treated by aspirin: a high prevalence of aspirin-resistance, however, has been observed in these subjects, inducing to investigate the mechanisms involved. Aspirin reduces platelet function both by decreasing the synthesis of Thromboxane A2 (TXA_2)and by increasing the synthesis on nitric oxide (NO), a powerful physiological anti-aggregating agent. In a recent study carried out in 45 healthy subjects,

we demonstrated that a short-time *in vitro* platelet exposure to high glucose reduces the aspirin anti-aggregating effect by decreasing the aspirin ability to increase NO synthesis, without influencing TXA_2 formation. Aim of this study is to evaluate whether oxidative stress is involved in this intriguing phenomenon.

Materials and methods: The study has been carried out in 8 healthy volunteers (M/F:4/4; age: 24.1±0.9 years; BMI: 22.58±0.4 kg/m^2), non smokers, with normal glucose tolerance and insulin sensitivity. In platelet-rich plasma (PRP) and washed platelets from venous blood samples, we evaluated the influence of a 30-min exposure to lysine acetylsalicylate (LAS: 5-300 micromol/l) on platelet aggregation induced by sodium arachidonate (NaAA; 1 mmol/l) and ADP (20 micromol/l) (Born's method), and on the NO synthesis (conversion of 3H-arginine to 3H-citrulline) without and with a 60 min pre-incubation with 25 mmol/l D-glucose, both in the absence and in the presence of the thiol antioxidant compound amifostine (200 micromol/l) added 20 min before LAS.

Results: High glucose reduced LAS ability to inhibit platelet responses agonists: actually, in PRP, Maximal Aggregation (MA) in response to NaAA was 70.4±2.9 without vs 84.7±2.3 with 25 mmol/l glucose (p<0.002) and MA in response to ADP was 64.5±1.4% without vs 90.1±4.0% with 25 mmol/l glucose (p<0.0001). Platelet exposure to amifostine did not modify platelet responses to agonists, but blunted the inhibitory effects exerted by high glucose on the LAS anti-aggregating action: in the presence of amifostine, MA in response to NaAA was 64.6±1.5 without and 67.1±1.8% with 25 mmol/l glucose (ns), MA in response to ADP was 56.8±2.1 without and 61.8±2.4% with 25 mmol/l glucose (ns). Furthermore, in washed platelets, high glucose inhibited the LAS-induced ability to enhance NO synthesis, which was (pmol/min/mg protein): 0.134±0.019 at baseline, 0.272±0.022 with LAS in the presence of 5 mmol glucose (p<0.001) and 0.127±0.026 with LAS in the presence of 25 mmol/l glucose (ns vs baseline). Washed platelet exposure to amifostine did not modify NO synthesis, but blunted the inhibitory effect exerted by high glucose on LAS-induced NO enhancements: in the presence of amifostine, NO synthesis (pmol/min/mg protein) was 0.142±0.005 at baseline, 0.318±0.02 with LAS in the presence of 5 mmol glucose (p<0.0001 vs baseline) and 0.211±0.013 with LAS in the presence of 25 mmol/l glucose (p<0.0001 vs baseline).

Conclusion: Oxidative stress is involved in the ability of high glucose to reduce the anti-aggregating effect of aspirin by impairing the aspirin-induced increase of NO synthesis. This information explains a potential mechanism involved in the aspirin resistance observed in diabetes.

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1325

The platelet-inhibitory effect of low-dose acetylsalicylic acid is dependent on glycaemic control in type 2 diabetes

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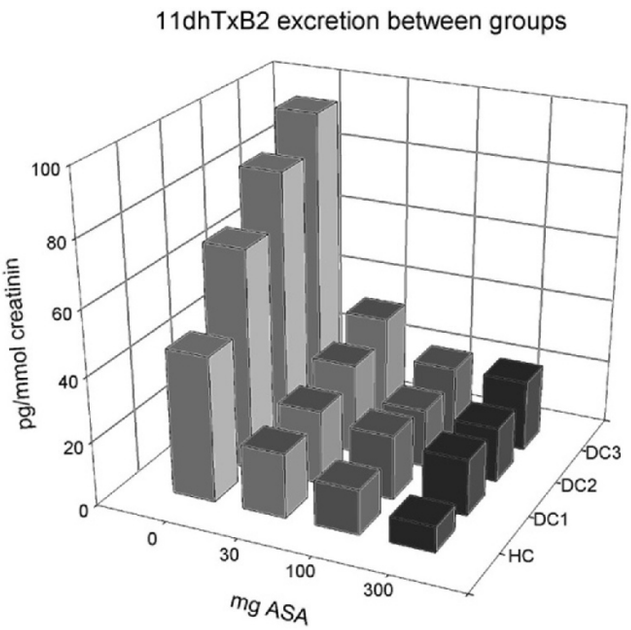
Background and aims: The clinical benefit of low-dose acetylsalicylic acid (ASA) treatment in primary prevention of cardiovascular events in type 2 diabetes (DM2) remains controversial. The platelet response to ASA has been suggested to be diminished in diabetes, but the role of hyperglycaemia has not yet been elucidated. In this study we determined whether (i) the baseline platelet activity and response to ASA differs in patients with DM2 as compared to healthy controls, (ii) glycaemic control influences the platelet response to ASA in DM2 and (iii) higher doses of ASA improve the platelet response in DM2.

Materials and methods: This is a prospective, single-centre, open-label trial. We aim to include 125 subjects and report here the preliminary data for 102 subjects. DM2 patients are categorized by HbA1c value < 7.0% (DC1: n=34), 7.0- 8.5% (DC2: n=33) and > 8.5% (DC3: n=22) and compared to healthy controls (HC: n=13). All subjects underwent three treatment periods, sequentially using 30 mg, 100 mg and 300 mg of ASA for ten days. Laboratory measurements were performed at baseline and following each treatment period. To assess the pharmacological efficacy of ASA, urinary 11-dehydrothromboxane B2 (11dhTxB2) was measured by ELISA. Platelet function was measured by optical platelet aggregation and Verify Now.

Results: Median baseline urinary 11dhTxB2 excretion was 45 pg/mmol (IQR 36-59) in HC, compared to 69 in DC1 (IQR 37-93), 84 in DC2 (IQR 47-101) and 95 in DC3 (IQR 73-143) (p=0.007, ANOVA). Treatment with ASA 30 mg significantly reduced 11dhTxB2 by 62%, 67%, 64% and 68% respectively. Absolute excretion remained significantly different between groups, and followed the same pattern as baseline (p=0.001). Subsequent treatment with 100 mg ASA further reduced 11dhTxB2 in DC2 (p<0.001) and DC3 (p=0.05) Increasing ASA to 300 mg resulted in a further 10% reduction in DC1 (p=0.026).Verify Now showed incomplete suppression of arachodonic acid(AA)-induced platelet aggregation at 30 mg ASA in all DM2 groups when compared to healthy controls (p=0.001). This difference became smaller with ASA 100 mg, with no further changes at 300mg. Interestingly, optical aggregation induced by 1 mmol/L AA was completely suppressed by 30 mg ASA in all groups, but a concentration of 2 mmol/L resulted in an escape from the ASA suppression in DC3.

Conclusion: Our results show that urinary 11dhTxB2 excretion as a measure of platelet activity is increased in DM2 and is highly associated with glycaemic control. ASA 30 mg only partially suppresses platelet activity in DM2, but ASA 100 mg is sufficient for adequate suppression of urinary 11dhTxB2 excretion.



Supported by: a NFCVE grant to BL

1326

***In vitro* platelet exposure to high glucose reduces the inhibitory effects exerted by chronic aspirin therapy on responses to agonists in non diabetic patients**

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Background and aims: We previously observed that "in vitro" incubation with high glucose of platelets from healthy subjects does not modify platelet responses to agonists but reduces the ability of aspirin added "in vitro" to inhibit platelet responses to agonists, suggesting that hyperglycaemia plays a role in the aspirin resistance described in diabetic patients. In the present study we investigated whether "in vitro" exposure of platelets to high glucose influences platelet responses to agonists in non diabetic patients on chronic aspirin treatment.

Materials and methods: We studied 56 non diabetic patients on chronic aspirin therapy (100 mg/day) owing to the presence of severe cardiovascular risk factors and/or previous cardiovascular events: M/F 33/23; age: 60.5±0.82 years; body mass index: 27.7±0.5 kg/m^2. Platelet sensitivity to aspirin was evaluated in platelet-rich plasma (PRP) by determining by Born's method Maximal Aggregation (MA) in response to Sodium Arachidonate (NaAA): patients were defined as "aspirin resistant" when MA in response to NaAA was greater

than 20%. In PRP from both aspirin sensitive and aspirin resistant patients, we evaluated by Borns' method platelet aggregation induced by 1 mmol/l NaAA, 10 µmol/l ADP, 4 µmol/l epinephrine and 4 mg/l Collagen in the presence or in the absence of a 60-min preincubation with high glucose (25 mmol/l).
Results: In aspirin sensitive patients (49/56), PRP incubation with high glucose significantly increased agonist-induced aggregation, being MA in response to NaAA 6.4±0.5% without vs 10.8±0.5% with 25 mmol/l glucose (p<0.0001), MA in response to ADP 67.7±4.8% without vs 77.0±4.7% with 25 mmol/l glucose (p<0.005), MA in response to epinephrine 24.7±3.0% without vs 28.0±4.1% with 25 mmol/l glucose (p<0.05), MA in response to Collagen 29.8±3.8% without vs 36.4±4.8% with 25 mmol/l glucose (p<0.006). The seven aspirin resistant patients presented higher baseline platelet responses to agonist vs the aspirin sensitive-ones: in particular, MA was: 30.4±3.0% vs 6.4±0.5% (p<0.0001) in response to NaAA ; 100.3±5.9% vs 67.7±4.8% (p<0.015) in response to ADP; 48.1±6.9% vs 24.7±3.0% (p<0.007) in response epinephrine; 49.6±2.5% vs 29.8±3.8% (p<0.05) in response to Collagen. In vitro exposure to high glucose of PRP of aspirin resistant patients did not modify responses to agonists, being MA without or with 25 mmol/l glucose 30.4±3.0% and 28.1±4.1% with NaAA (ns); 100.3±5.9% and 98.6±7.9% with ADP (ns); 48.1±6.9% and 46.7±7.6% with epinephrine (ns); 49.6±2.5% and 54.0±9.3% with collagen (ns).
Conclusion: In aspirin sensitive non diabetic patients on chronic aspirin treatment, high glucose added "in vitro" increases platelet responses to agonists, demonstrating that it partially overcomes the inhibitory effect of aspirin on platelet responses with direct effects on platelets, adding another piece of information on the role of hyperglycaemia in the reduction of aspirin sensitivity in diabetes mellitus. The lack of this glucose effect in aspirin resistant patients indicates that when platelets are already aspirin resistant the modulating effect of glucose on platelet sensitivity to aspirin disappears.
Supported by: an EFSD/sanofi-aventis grant

1327

Fibrin clot structure characteristics in type 2 diabetes: relationship with cardiometabolic risk factors
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Background and aims: Atherothrombotic complications are the main cause of mortality and morbidity in individuals with diabetes. A compact fibrin network structure with increased resistance to fibrinolysis has been documented in individuals at high risk of cardiovascular disease. The aim of the present work was to study the relationship between fibrin clot structure and fibrinolysis and cardiometabolic risk factors and a history of ischemic heart disease (IHD) in a large cohort of type 2 diabetes (T2DM) subjects.
Materials and methods: Using a previously validated turbidimetric assay, clot structure and fibrinolysis were assessed in 875 individuals with T2DM (mean age 68 (CI 67.7-68.3) 450 males) enrolled on the Edinburgh T2DM study. The following parameters were analysed: maximum absorbance (MA), a measure of clot density, time from start to full clot formation (CT), an indicator of clotting potential and lysis time (LT) from full clot formation to 50% lysis, assessing the efficacy of the fibrinolytic pathway. We also measured cardiac inflammatory markers including C-reactive protein (CRP) and complement C3 levels using ELISA techniques.
Results: Female patients had denser clots than males (MA=0.37±0.005 and 0.34±0.004 au respectively, p<0.01) and CT was longer (567.1±6.6 and 522±5.6 sec respectively, p<0.01). LT was prolonged in female subjects at 803.1±19.8 sec compared with males (664.7±12.1 sec; p<0.01). Males with a previous history of IHD had a weak increase in MA (0.35±0.008 and 0.33±0.006 au IHD and non-IHD, respectively, p<0.05), a relationship not seen in female subjects. We analysed the effects of aspirin therapy which was associated with an increase in LT (755±16.1 sec) compared to those not on aspirin (662±12.7 sec, p<0.05). The longer lysis time in aspirin-treated subjects was still evident after excluding individuals with a history of IHD.C-reactive protein (CRP) correlated with MA in both females and males (r=0.24 and 0.27, respectively; p<0.01), whereas C3, which is incorporated into the fibrin network, correlated with LT in both females and males (r=0.25 and r=0.27, respectively; p<0.01). HbA1c showed a weak correlation with MA and LT in male subjects only (r=0.10 and 0.10, respectively, p<0.05), whereas estimated glomerular filtration rate negatively correlated with MA in both males and females (r=0.18 and 0.29 respectively; p<0.01).
Conclusion: i) female patients with diabetes have a more thrombotic clot structure which may contribute to loss of cardiovascular protection in this group. ii) aspirin treatment in diabetes is associated with a paradoxical increase in clot lysis time, suggesting one mechanism for the reduced clinical efficacy of this agent in diabetes. iii) clot structure in T2DM subjects is related to inflammatory and metabolic parameters.
Supported by: King Fahad Medical City and NIHR(UK)

1328

Abnormal glucose tolerance in atrial fibrillation and impact on inflammation, endothelial/platelet function, fibrinolysis, extracellular matrix metabolism and NT-pro-BNP
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Background and aims: Although an abnormal glucose tolerance (AGT), i.e. diabetes mellitus (DM) or pre-DM, increases the risk for atrial fibrillation (AF) few studies have assessed the extent to which AGT modulates CV risk factors/parameters in patients with AF.
Materials and methods: In a case control study amongst 75-year old subjects with AF or in sinus rhythm (all previously undiagnosed with DM or pre-DM), we examined the prevalence of undiagnosed AGT (by a 75-g oral glucose tolerance test [OGTT] classified according to World Health Organisation criteria) and explored its association to AF duration as well as to circulating CV risk biomarkers of inflammation (C-reactive protein [CRP], Interleukin [IL]-6), endothelial/platelet function (Monocyte chemotactic protein [MCP]-1, P-selectin, CD-40 ligand), fibrinolysis (tissue plasminogen activator antigen [tPAag], plasminogen activator inhibitor-1 [PAI-1] activity), extracellular matrix metabolism (matrix metalloproteinase [MMP]-9, tissue inhibitor metalloproteinase [TIMP]-1) and ventricular function (N-terminal fragment pro-brain natriuretic peptide [NT-pro-BNP]). Between group comparison was conducted non-parametric (Kruskal-Wallis).
Results: Prevalence of undiagnosed DM among the 108 subjects (male/female 73/35, BMI 25.4±3.2) in sinus rhythm and the 46 (male/female 34/12, BMI 25.3±3.7) with AF (median AF duration 5 years) where 3.7% and 13.0%, respectively (p=0.031). Patients with AF duration ≥ 5 years had a higher prevalence of DM and pre-DM (61.1%) as compared to AF duration < 5 years (25%, p=0.0014) or no AF (39.0%, p=0.17). Patients with AF duration ≥ 5 years also had elevated levels of IL-6 (4.27±3.10, p=0.017), MCP-1 (329±82, p= 0.004), CD40 ligand (135±196, p=0.026), PAI-1 activity (16.0±9.5, p=0.004), TIMP-1 (165.6±26.3, p=0.008) and NT-ProBNP (1023±1212, p< 0.001) as compared to those with AF < 5 years (IL-6: 2.89±1.42, MCP-1: 295±85, CD40 ligand: 77±73, PAI-1 activity: 13.7±8.3 TIMP-1: 142.9±20.7, NT-Pro-BNP 741±630) or no AF (IL-6: 2.80±1.85, MCP-1: 290±202, CD40 ligand: 91±95, PAI-1 activity: 10.1±6.7, TIMP-1: 156.1±27.4, NT-Pro-BNP: 186±353). A dosage related, and in part synergistic, impact on the CV risk biomarker levels of AF in the presence of AGT was seen for most parameters assessed; with statistical significance reached for MCP-1, PAI-1 activity and NT-ProBNP (table 1).
Conclusion: Undiagnosed dysglycaemia is prevalent in long standing AF and CV risk biomarkers were adversely modulated by the presence of both AF and AGT. The aggravated CV biomarker profile seen in patients with both AF (especially if long standing) and AGT may play a causal role for the increased CV morbidity and premature mortality observed in this patient group and calls for further attention and research.

Table 1. Levels of circulating CV risk biomarkers according to AF and OGTT status.

	Sinus rythm + normoglycaemia (n=61)	AF + normoglycaemia (n=28)	Sinus rythm + AGT (n=47)	AF + AGT (n=18)	p-value (Kruskal-Wallis)
CRP (mg/L)	3.2±2.9	3.4±3.0	3.9±3.7	3.5±2.9	0.865
IL-6 (pg/mL)	2.64±1.63	2.94±1.19	3.01±2.11	4.19±3.30	.0126
MCP-1 (pg/mL)	311±261	288±70	262±65	339±98	0.003
P-selectin (ng/mL)	31.6±12.3	31.1±9.3	33.8±9.7	32.8±10.3	0.455
CD40 ligand (pg/mL)	88±50	81±72	96±133	128±198	0.263
tPAag (ng/mL)	15.7±4.2	15.5±6.4	15.0±4.4	17.5±5.6	0.412
PAI-1 activity (U/mL)	8.9±5.7	12.6±8.2	11.5±7.7	17.6±8.9	<0.001
MMP-9 (ng/mL)	204.9±98.9	203.0±106.0	199.5±95.9	168.7±74.0	0.493
TIMP-1 (ng/mL)	153.4±26.9	146.5±21.9	159.6±28.0	160.0±28.6	0.148
NT-ProBNP (pg/mL)	114±83	720±561	281±515	1117±1255	<0.001
HOMA-IR (%)	3.8±1.6	4.1±3.1	4.3±2.1	6.1±5.6	0.505

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Plasminogen activator inhibitor-1 and thrombin activable fibrinolysis inhibitor in patients with type 2 diabetes

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Background and aims: Hypofibrinolysis is a common finding in patients with diabetes mellitus and risk factor for developement of cardiovascular disease. The present study was undertaken to assess the plasma levels of two main inhibitors of fibrinolysis - plasminogen activator inhibitor (PAI-1) and thrombin activable fibrinolysis inhibitor (TAFI) and their relation with clinical and metabolic parameters in diabetic patients with and without vascular complications.
Materials and methods: The study was carried out on 53 patients with type 2 diabetes mellitus (25 women, 28 men). In the examined group 21 patients had coronary heart disease (CHD), 22 hypertension and 17 diabetic rethinopathy. The control group comprised 24 healthy subjects matched for sex and age. The PAI-1 level was measured using Asserachrom PAI-1 set and TAFI by means of Imuclone TAFI ELISA.
Results: In comparison to the healthy controls diabetics revealed significantly higher PAI-1 (27,7±16,4 vs 55,3±29,9 ng/ml, p<0,0001) but lower TAFI levels (115,2±24,0 vs 87,3±20,3%, p<0,0001). The diabetics with CHD and hypertension but not with rethinopathy had significantly increased level of PAI-1 in comparison to the patients without these complications (67,8±33,7 vs 47,1±24,3 ng/ml, p=0,02; 67,0±30,8 vs 47,0±26,7 ng/ml, p=0,01; 57,3±27,5 vs 54,4±31,3 ng/ml, p>0,05, respectively). We found significant positive correlations between the levels of PAI-1 and BMI as well as the levels of triglicerides and negative correlations between PAI-1 and plasmin-antiplasmin complexes (PAP). There were no significant correlations between PAI-1 levels and the levels of HbA1c and GFR. There were no significant differences in the mean levels of TAFI in diabetics with CHD, hypertension and rethinopathy and those without these complications. No significant correlations between TAFI levels and BMI, lipids, HbA1c, PAP and GFR were found.
Conclusion: The data prove the important role of PAI-1 - but not TAFI - in the impairment of fibrinolysis and the developement of cardiovascular complications in patients with type 2 diabetes mellitus.

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PAI-1 is an independent maker of metabolic disorders in young adults born small for gestational age

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Background and aims: Insulin resistance, metabolic syndrome (MS) and cardiovascular diseases have been associated with being born small for gestational age (SGA). However, the mechanisms underlying these associations are still unknown. Fibrinolysis is regulated by plaminogen activator inhibitor type-1 (PAI-1), secreted by the adipose tissue. Increased PAI-1 levels predispose to the development of atherosclerotic plaques prone to rupture. In epidemiological studies PAI-1 levels have been associated with MS and identified as a predictive factor for myocardial infarction. Few studies have examined these associations in subjects born SGA.
Materials and methods: The study population is made of a community-based cohort of young adults, mean age 29.4 years, selected on their birth characteristics. 557 adults born SGA (birth weight under the 10th percentile) were compared to 671 subjects born appropriate for gestational age (AGA) (birth weight between 25th and 75th percentiles). MS was defined using the WHO definition. PAI-1 activity was measured in citrated plasma with a bio immunoassay.
Results: BMI (24.1 ± 4.4 vs 24.2 ± 5.3 kg/m^2) was similar between in AGA and SGA whereas body fat (22.0 ± 8.2 vs 23.2 ± 9.0 %) was significantly increased in the SGA group, p=0.01. MS was more prevalent in the SGA group (8.7%) versus AGA group(5.5%), p=0.03. PAI-1 concentration was correlated with waist circumference, plasma triglycerides, HOMA-IR and associated with male gender and MS in both groups. After adjustment on these variables, PAI-1 concentrations remained significantly increased in the SGA group (12.2 ± 21.2 UI/ml vs 10.0 ± 13.5 UI/ml, p=0.01). PAI-1 concentration above 4.9 UI/ml (median of PAI-1 concentration in the AGA group) was present in 94% of the subjects with MS. Moreover, OR for having elevated PAI-1 was 1.48 [1.08; 1.95] in the SGA group (p=0.005).
Conclusion: PAI-1 plasma concentrations were significantly increased in SGA subjects independently of MS. These data suggest that elevation of PAI-1 concentrations could be taken as an indication of an abnormal secretion at the level of the adipose tissue and could consequently be implicated in the development of the metabolic disorders reported in SGA subjects.

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Soluble receptor for advanced glycation endproducts and its inflammatory ligands EN-RAGE and HMGB1 in type 1 and type 2 diabetes mellitus

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Background and aims: The aim of the study was to compare concentration of soluble receptor for advanced glycation endproducts (sRAGE) and its natural pro-inflammatory ligands, EN-RAGE (extracellular newly identified RAGE-binding protein, S100A12) and HMGB1 (high mobility group box-1) with diabetes control, albuminuria, cell adhesion molecules and von Willebrand factor (vWF) in Type 1 (T1DM) and Type 2 (T2DM) diabetes mellitus.
Materials and methods: Total number of 45 T1DM (age 47±13 yrs, diabetes duration 21±12 yrs) and 68 T2DM (age 64±10 yrs, diabetes duration 12±9 yrs) were examined. Control group consisted of 41 healthy persons of comparable age. Serum concentrations of sRAGE, EN-RAGE, HMGB1, ICAM-1, VCAM-1, P-selectin, E-selectin and vWF have been determined by ELISA kits. HbA1c was estimated by HPLC and albuminuria by radioimmunoassay. Both groups of T1DM and T2DM were divided according to microalbuminuria (MA) to MA+ subgroup with MA > 3 g/mol creatinine and MA- subgroup with MA < 3 g/mol creatinine.
Results: Serum sRAGE concentration was significantly higher in both T1DM (1137±78 μg/l, p<0,001) and T2DM (995±63 μg/l, p<0,001) compared to healthy persons and it was higher in MA+ than in MA- subgroups. In T1DM the RAGE ligand (HMGB1 and EN-RAGE) concentrations were more elevated in MA- than in MA+ subgroup in which levels were similar to controls. Significant positive relationship was found between sRAGE and HbA1c (r=0.36, p<0.01), diabetes duration (r=0.58, p<0.01), von Willebrand factor (r=0.36, p<0.005) and albuminuria (r=0.43, p<0.001) in T2DM. sRAGE levels were not related to the above parameters in T1DM, but they significantly correlated with ICAM (r=0.39, p<0.01) and VCAM (r=0.63, p<0.0001). Positive relationship was found between HMGB1 and MA (r=0.79, p<0.05), ICAM (r=0.84, p<0.05) and E-selectin (r=0.79, p<0.05) in MA+ T1DM. No relationship was observed between sRAGE and its ligands.
Conclusion: Serum sRAGE concentration reflects protective ability against AGEs created more significantly in diabetes with increased albuminuria. It corresponds to higher sRAGE levels in these patients. Our results demonstrate differences in RAGE ligands between T1DM and T2DM. Their higher levels found in younger T1 and T2 diabetic patients with other risk factors compared to older patients with already established micro- and macrovascular disease are suspicious from the higher activity promoting RAGE ligand creation in former patients. Their role as markers in development of chronic vascular complications will be evaluated in the follow-up study.

Results:

	T1DM		T2DM		Controls
	MA- (n = 38)	MA+ (n = 7)	MA- (n = 50)	MA+ (n = 18)	(n = 41)
HbA1c (%, IFCC)	7.40^{a}±0.22	8.33^{a}±0.25	6.43^{a}±0.24	8.10^{a}±0.69	3.60±0.20
MA (g/mol creat.)	1.15±0.14	12.04^{a}±5.35	1.27±0.15	47.94^{a}±11.51	0.80±0.10
HMGB1 (μg/l)	2.45^{by}±0.27	1.02±0.27	2.58^{a}±0.29	2.65^{a}±0.42	0.95±0.14
sRAGE (μg/l)	1078±89	1461^{a}±117	877±55	1322^{ay}±162	824±48
EN-RAGE (μg/l)	277^{ax}±33	125±33	266^{a}±28	365^{a}±53	110±10
vWF (μg/l)	110±7	135^{a}±25	109±5	177^{ay}±17	88±32
ICAM (μg/l)	249^{b}±12	320^{b}±42	285^{b}±26	325^{b}±35	206±10
VCAM (μg/l)	863^{a}±72	1194^{a}±147	796^{a}±39	991^{a}±107	365±11

Results are mean±SEM, significant difference to controls: $^{a}p<0.001$, $^{b}p<0.01$, and between MA+ and MA-: $^{x}p<0.05$, $^{y}p<0.01$.

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Intermittent high glucose promotes expression of proinflammatory cytokines in monocytes

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Background and aims: Diabetes confers an increased propensity to atherosclerosis. Postprandial hyperglycemia seems to impose more deleterious effects on vessels. The aim of this study was to examine expression of proinflammatory cytokines from monocytes under fluctuating glucose condition.
Materials and methods: Monocytic cells (THP-1) were divided into four groups and cultured in the presence of 5mmol/l or 15 mmol/l glucose and in a fluctuating condition(12h exposure to 15mmol/l glucose or mannitol medium followed by 12h exposure to 5 mM glucose or mannitol medium) respectively.Concentrations of IL-6 and TNF-α in the supernatants and CD11b MFI(mean fluorescence intensity) in monocytes surfaces were measured after 72h' culture.
Results: Monocytes exposed to fluctuating glucose condition expressed highest levels of IL-6,TNF-α and CD11b, and in the second place were monocytes exposed to fluctuating mannitol condition. Monocytes cultured in 15mmol/l glucose medium expressed a lower level of cytokines than those cultured in such fluctuating conditions, but a higher level compared with those in 5mmol/l glucose medium (for IL-6: 204.99±25.08 pg/ml, 179.97±37.14 pg/ml,151.61±21.82 pg/ml and 122.41±18.19 pg/ml respectively; for TNF-α:148.73±15.71 pg/ml, 131.46±16.67 pg/ml, 96.91±15.14 pg/ml and 74.08±7.46 pg/ml respectively; for CD11b MFI: 77.73±7.51,68.75±4.01, 61.58±3.05 and 53.82±6.68 respectively). Differences of these cytokines among the four groups were statistically significant.
Conclusion: The results indicate that exposure to fluctuating glucose concentrations enhances activation of monocytes compared with stable elevations in glucose concentration. The effects are partly attributable to the inherent osmotic changes.These findings indicated that reducing fluctuations in circadian glucose concentrations has important implications for the treatment strategies in diabetic patients with macrovascular complications.

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Association of urine adiponectin levels and marker of endothelial damage in type 2 diabetes without microalbuminuria

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Background and aims: Urinary albumin excretion (UAE) and intima media thickness (IMT) of the carotid artery are considered subclinical markers of endothelial cell damage preceding atherosclerosis in type 2 diabetes. Recent studies reported that Urine (U-) adiponectin was significantly increased in type 2 diabetes. U-adiponectin reflects early vascular damage in a more efficient way compared to serum adiponectin, which is easily influenced by metabolic changes. Our observational study assessed whether there is an association between U-adiponectin and UAE, change of IMT at baseline and after 1 year in type 2 diabetes without microalbuminuria.
Materials and methods: We enrolled 90 (37 men, 53 women) type 2 diabetic patients without microalbuminuria who presented with good glycemic control for 1 year. Total plasma (P-) and U-adiponectin were determined by enzyme-linked immunosorbent assay kit. U-adiponectin levels were adjusted for urinary creatinine excretion. The measurements were performed on both common carotid arteries avoiding areas of atherosclerotic plaque formation, and the mean IMT was used in this study.
Results: Baseline characteristics of the patients were: age 58.7 ± 8 years, mean body mass index (BMI) 25.1 ± 3.1 kg/m^2, HbA1c value 6.9 ± 1.2 %, and UAE 8.2 ± 6.8 ug/mg creatinine. The mean duration of diabetes was 9.5 years. BMI, HbA1c and triglyceride levels were not significantly different between baseline and at 1 year (p=.160, p=590, p=.418, respectively). Mean IMT was 0.70 ± 0.1 mm at baseline, and slightly increased to 0.75 ± 0.1 mm after 1 year, with a statistically significant difference (p<0.001). Baseline P-adiponectin and U-adiponectin were 13.0 ± 15.7 ug/mL, 4.7 ± 6.8 ug/mg creatinine,

respectively, and at 1 year, 11.0 ± 11.4 ug/mL, 4.8 ± 6.5 ug/mg creatinine, not demonstrating a significant difference (p=.226, p=.913, respectively). We analyzed the two groups, one with the increased IMT at 1 year (n=53) compared to the baseline IMT and the other with the decreased IMT at 1 year (n=31). HbA1c was significantly higher in the increased IMT group compared to the decreased IMT group (7.1 ± 0.1 % vs 6.6 ± 0.2 %, p=.039), while BMI, blood pressure and lipid levels were not significantly different between the two groups. U-adiponectin was higher in the increased IMT group than in the decreased IMT group, although there was no significant difference (5.1 ± 6.9 ug/mg creatinine vs 3.2 ± 4.6 ug/mg creatinine, p=.133). U-adiponectin revealed a significant positive correlation with UAE (r=.259, p=.014), while it was not significantly correlated with IMT (r=-.100, p=.350) in our study subjects.
Conclusion: Our study implies that U-adiponectin levels may reflect early glomerula vascular damage in the pre-albuminuric level in type 2 diabetic patients. On the other hand, U-adiponectin may not be associated with macrovascular damage. The exact mechanism and association between U-adiponectin and vascular damage need further investigating.
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Formation of methionine-sulfoxide at position 1606 of Von Willebrand Factor inhibits cleavage by ADAMTS-13: a new prothrombotic mechanism promoted by oxidative stress in diabetes

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Background and aims: An enhanced formation of reactive oxygen species and peroxynitrite occurs in diabetes. Peroxynitrite oxidizes methionine and tyrosine residues to methionine sulfoxide (MetSO) and 3-nitrotyrosine (NT), respectively. Notably, ADAMTS-13 cleaves von Willebrand factor (VWF), which bind and activate platelets in the microcirculation, exclusively at the Tyr1605-Met1606 peptide bond. We hypothesized that peroxynitrite could oxidize either or both of these amino acid residues, thus potentially affecting ADAMTS-13-mediated cleavage.
Materials and methods: Nonsmokers-healthy subjects (n=13, age 38-55), without risk factors for cardiovascular disease and 16 age- and sex-matched subjects with type 2 DM (T2DM) not on chronic medications including NSAIDs, vitamin E, or statins, were consecutively enrolled. The plasma level of VWF was measured as antigen and ristocetin cofactor. Moreover, we tested our hypothesis using synthetic peptide substrates based on: (1) VWF Asp1596-Ala1669 sequence (VWF74) and (2) VWF Asp1596-Ala1669 sequence containing nitrotyrosine (VWF74-NT) or methionine sulfoxide (VWF74-MetSO). The peptides were treated with recombinant ADAMTS-13 and the cleavage products analyzed by RP-HPLC. As adjunctive analysis, in 15 type 1 diabetic subjects we explored the relationship between the 24hrs-glycemic variability (obtained from Continuous Glucose Monitoring System and measured with CONGA index) and total carbonyls (a surrogate marker of oxidative stress) and ADAMTS-13 activity.
Results: T2DM subjects showed a significantly increased plasma level of VWF (p<0.001). The carbonyl content of VWF (a marker of oxidative modification of the protein) was significantly higher in T2DM than in controls (p<0.01). Moreover, compared to VWF purified from control subjects, VWF preparations from T2DM patients showed a relative resistance to ADAMTS-13 hydrolysis. In particular, VWF74 oxidized by peroxynitrite underwent a severe impairment of its hydrolysis. Likewise, VWF74-MetSO was minimally hydrolyzed, whereas VWF74-NT was hydrolyzed slightly more efficiently than VWF74. Oxidation of purified VWF multimers did not alter their electrophoretic pattern nor their ability to induce platelet aggregation by ristocetin. Furthermore, we found a strong and positive linear correlation between CONGA1 and total carbonyls and ADAMTS-13 activity (P=0.013, R=0.904; P=0.003, R=0.952, respectively).
Conclusion: Overall, these findings indicate that oxidative stress and glycemic variability may contribute to prothrombotic effects, hindering the proteolytic processing by ADAMTS-13 of high-molecular-weight VWF multimers.

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Association of serum endothelin-1 with insulinaemic status in prediabetic and newly diagnosed type 2 diabetic subjects

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Background and aims: Endothelin-1 (ET-1), an atherogenic marker, has been shown to be raised in T2DM subjects. However, the factors contributing to the elevation of ET-1 in T2DM is still unclear. Since IFG and IGT are intermediate stages in the development of diabetes, we have studied ET-1 in relation to glycaemic and insulinemic status in these prediabetic groups to get better insight on this issue.
Materials and methods: A total number of 160 subjects of Bangladeshi origin, consisting of 18 IFG, 47 IGT, 46 newly diagnosed T2DM patients and 49 healthy subjects were included in the study. Glucose was estimated by glucose-oxidase, lipids by enzymatic- colorimetric, and insulin and endothelin-1 were estimated by enzyme linked immunosorbent assay (ELISA). Results were expressed as mean±SD and appropriate tools were used in statistical comparison.
Results: Age and BMI were matched among all the groups. Absolute insulin (μIU) level in IGT and T2DM were significantly higher compared to the controls (p<0.001 for both). HOMA%B (mean±SD) was significantly lower in IFG and T2DM groups (p=0.004 and <0.001) and higher in IGT (p=0.006) group compared to controls. HOMA%S was significantly lower in IGT and T2DM groups (p=0.002 and 0.003 respectively) compared to the Control, but it did not show any significant difference between IFG and Control groups. Lipid levels were almost similar in all three groups compared to the Control except significantly higher triglyceride and total cholesterol level in T2DM groups (p=0.001 and p=0.024 respectively). Mean value of serum endothelin-1 was 6.85±4.4, 10.34±4.7, 10.14±6.8 and 10.48±5.7 in the Control, IFG, IGT and T2DM subjects respectively. This atherogenic marker was found to be significantly higher in prediabetic (IFG: p=0.009 and IGT: p=0.006) and T2DM (p=0.001) groups. On Spearman's correlation analyses ET-1 showed association with fasting glucose in T2DM subjects, although it did not show any significant correlation with HOMA%S or HOMA%B in the prediabetic and T2DM groups. Multinomial logistic regression analyses showed that ET-1 was associated with all the three hyperglycemic groups (IFG: p=0.005; IGT: p=0.007 and T2DM: p=0.003) when adjusted for age, WHR and gender.
Conclusions: Endothelin-1 is raised in hyperglycemic conditions regardless the stages of its natural history and this elevation is directly associated with hyperglycemia rather than insulin deficiency or insulin resistance.
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Inducible Nitric Oxide Synthase (iNOS)regulation by Ca^{2+}/Calmodulin-dependent protein kinase II in vascular Smooth Muscle Cells (vSMCs) from diabetic rats

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Background and aims: In diabetes, increased cytokine plasma levels could induce iNOS expression contributing to vascular damage. In addition to transcriptional regulation, iNOS activity may be posttranslationally regulated by palmitoylation and intracellular trafficking. It has been recently demonstrated that the multifunctional protein kinase CaMKII, a known contributor to vascular dysfunction in diabetes, may also play a significant role in iNOS-specific trafficking and activity following cytokine induction. Thus, the aim of the present study was to investigate the relationships between cytokine increased iNOS activity and Ca^{2+}/CaMKII/CaMKIIdelta2 pathway involvement in vSMCs from diabetic rats (DR).
Materials and methods: We measured iNOS expression (RT-PCR, Western Blot) and activity (conversion L-(3H)-arginine into L-(3H)-citrulline), intracellular Ca^{2+} levels (fluorescence video imaging), CaMKII phosphorylation

(Western Blot), iNOS/CaMKIIdelta2 co-immunoprecipitation (Western Blot) and nitrotyrosine levels (immunofluorescence) in cultures of aortic vSMCs from 10 diabetic (90% pancreatectomy, DR) and 10 control (sham surgery, CR) rats, after 24 hrs incubation with 20 μg/ml Lipopolysaccharide (LPS).
Results: LPS increased iNOS expression to the same extent in CR and DR, while iNOS activity was about 7 folds greater in DR. As to the effect of LPS on increased Nitric Oxide (NO) production in DR, exposure to LPS led to an increase of intracellular calcium levels (1.9 folds increase) and Ca^{2+}-dependent CaMKII phosphorylation (3 folds increase) followed, as expected, by the absence of iNOS/CaMKIIdelta2 co-immunoprecipitation. These LPS effects were associated to the greater induced iNOS-specific activity in DR. In CR, instead, LPS failed to affect these parameters, which were not different from basal levels after LPS stimulation. In addition, the increased iNOS activity in DR was accompanied by a significant increase in intracellular nitrotyrosine levels (1.5 folds increase).
Conclusion: In conclusion, as compared to CR vSMCs, DR cells showed a greater LPS induced iNOS activity associated to an activation of Ca^{2+}/CaMKII/CaMKIIdelta2 pathway. In addition, as we have previously shown, DR cells exhibit in this culture conditions a marked increase in O^{2-} levels. Thus, the LPS increased NO release in diabetic pro-oxidant milieu gets converted into peroxinytrite and then enhanced nitrotyrosine generation. Together these results indicate for the first time that signal pathway involving CaMKII contributes to increase iNOS activity and nitrotyrosine generation in diabetes and might provide new insight in the mechanisms linking diabetes and atherosclerosis.

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Multifactorial risk factor intervention in type 2 diabetic patients significantly improves global arginine bioavailability ratio
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Background and aims: Endothelial dysfunction is defined by reduced bioavailability of nitric oxide (NO) and has been shown to be associated with increased cardiovascular risk. The unique source for NO-synthesis is arginine and ornithine and citrulline are the products of arginine metabolism. Global arginine bioavailability ratio (GABR) is defined as arginine/(ornithine + citrulline). Recently published data showed an inverse association between GABR and cardiovascular events. The aim of our study was to investigate the impact of a multifactorial risk factor intervention on GABR as a cardiovascular surrogate parameter in type 2 diabetic patients.
Materials and methods: We investigated type 2 diabetic patients, that did not reach treatment targets according to current local guidelines in two out of three oft the following parameters: HbA1c <6,5%, LDL-cholesterol <100 mg/dl or blood pressure <130/80 mmHg. GABR was measured at baseline and after 3 months. During this time therapy was intensified according to current guidelines aiming to reach the treatment targets. Arginine, ornithine and citrulline were chromatographically determined after precolumn-derivatisation followed by fluorescent detection. Intima media thickness was measured by B-mode ultrasound.
Results: We investigated 41 patients (25 males/16 females) with a mean age of 60±10 years. Baseline characteristics and treatment outcomes are shown in table 1. Intensified risk factor management significantly improved GABR (0,33±0,12 at baseline vs. 0,38±0,14 after 3 months; p=0,018). A significant improvement was only seen in patients with short diabetes duration (<5 years) whereas in patients with longer diabetes duration improvement did not reach statistical significance. A linear model including the duration of diabetes, the change of LDL-cholesterol, change of HbA1c, change in blood pressure turned out diabetes duration as the only significant predictor (p=0.049) of GABR improvement. Furthermore the change of GABR was inversely correlated with mean intima media thickness (r=-0,381, p=0.014).
Conclusion: In type 2 diabetic patients intensified risk factor intervention improves global arginine bioavailability ratio as a parameter of endothelial function. Duration of diabetes seems to be an important factor influencing the capacity of GABR improvement.

Treatment outcomes

	Baseline	3 months	p-value
sex (m/f)	25/16		
age (years)	60±10		
Duration of diabetes (years)	8±7		
LDL cholesterol (mg/dl)	108±52	79±48	<0,001
HDL cholesterol (mg/dl)	46±13	50±16	<0,001
HbA1c (%)	8,5±1,2	7,9±1,4	<0,001
RR systolic (mmHg)	151±17	131± 23	<0,001
RR diastolic (mmHg)	89±9	77± 14	<0,001

Supported by: MSD, AESCA, AstraZeneca, Takeda, Novo Nordisk

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Formation of methylglyoxal-adducts in plasma is associated with LDL and triglyceride level: implications for diabetic atherosclerosis
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Background and aims: Protein glycation leading to AGE is enhanced in diabetes by increases in blood glucose *per se,* and collaterally, by endogenous production of reactive carbonyls. Low weight α-dicarbonyls are formed as glycolytic intermediates during metabolic conversion of glucose and/or during lipid peroxidation. Among AGE precursors, methylglyoxal (MG) is considered as one of the key intermediates. We hypothesized it to be a common product of both carbonyl and oxidative stress, and investigated the MG biogenesis in relation to glycemic and lipid status in diabetic patients.
Material and methods: Serum and urine MG-adduct content was measured by DELFIA method in 83 diabetic patients and 20 controls. Fasting (FG) and postprandial (PPG) glucose level, HbA1c, LDL and HDL cholesterol, plasma triglyceride and homocysteine level were determined along with routine biochemical parameters.
Results: Significant positive relationship was observed between serum level of MG-adducts and LDL (r=0.31 p=0.003) whereas fasting glucose correlated inversely (r= -0.33 p=0.001) as well as PPG (r= -0.23 p=0.041) and HbA1c (r= -0.22 p=0.036). Similarly, significant correlations were also found between urinary levels of MG-adducts and postprandial glucose (r= -0.28 p=0.023), serum triglycerides (r=0.31 p=0.003), homocystein (r=0.57 p=0.0007), HDL (r= -0.28 p=0.007) and urine albumine/creatinine ratio (r=0.53 p=0.002). Stepwise linear regression was performed using serum or urine MG-adducts as dependent variable and HbA1c, fasting and postprandial glucose, LDL, HDL, triglycerides, serum creatinine, homocystein and urine albumine/creatinine ratio as independent variables. Of these, only LDL-cholesterol (regression coefficient=0.29) and FG (regression coefficient=-0.28) were independent predictors of MG-adducts in serum (p<0.00046), whereas urine albumine/creatinine ratio, PPG, and triglycerides were independently associated with their urine content (p<0.0062). LDL-cholesterol >3.0 mmol/L discriminate patients who had a higher serum level of MG-adducts (median (10^{th} and 90^{th} percentile) 465 (251-1254) *vs* 331(169-706) mgEq/L, p=0.0052), although there was no between-subgroup difference in glycemic control. Patients on statin treatment had lower MG-adducts although the difference did not reach statistical significance. The positive relationship between LDL-c and MG-adducts (r=0.38, p=0.042) was noted in the patients (n=54) free of statin treatment, whereas in the statin-treated subgroup, there was an inverse tendency (r=-0.28, p=0.83) but no significant yet. A significant correlation between homocysteine and urinary excretion of MG-adducts (r=0.8; p=0.02) was recorded in patients with a history of macrovascular disease.
Conclusion: A highly significant relationship between LDL and MG-adduct production, as well as tight correlation between triglycerides and urinary MG-adduct excretion suggest that lipoxidation and glyceraldehyde-3-phosphate route, along with the glycolytic pathway, might be an important source of MG generation. The glycotoxin methylglyoxal seems to be a common factor linking the two dominant metabolic changes in diabetes, hyperglycemia and intensive lipolysis, with vascular pathobiochemistry of diabetes.

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Vitamin B1 analogue benfotiamine improves survival and proliferation of diabetic or high glucose challenged resident cardiac stem cells

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Background and aims: Diabetes mellitus (DM) is a potent and prevalent risk factor for coronary artery disease and heart failure. Furthermore, DM directly impinges on the heart's function by accelerating the aging of cardiomyocytes and resident cardiac progenitor cells (CPCs). Treatments able to prevent CPC senescence and thereby preserve heart reparative capacity are urgently needed. Our earlier studies demonstrated the ability of vitamin B1 analog benfotiamine (BFT) to prevent the development of diabetic cardiomyopathy. Here, we aimed to verify whether BFT may benefit the survival, proliferation and differentiation of resident CPCs.
Methods and results: Sca-1$^+$ CPCs were extracted from left ventricle of STZ induced type-1 DM mice after 20 weeks of DM induction using commercially available kit. Flowcytometric analysis revealed marked reduction in the number (23±4% vs 12±2%, P<0.01) and increased apoptosis (28±3% vs 10±4%, P<0.001) of CPCs from diabetic mice compared to age-matched healthy controls (n=7 mice per group). When cultured under normal glucose (5%) conditions, diabetic CPCs showed increased apoptosis and reduced protein expression of the cell survival Pim-1/Bcl-2 signalling pathway and significantly lost their ability to differentiate into cardiomyocytes when exposed to differentiation medium. However, treating diabetic animals with BFT (70mg/kg/day, for 16 weeks), markedly inhibited the apoptosis of CPCs through activation of Bcl-2 and phosphorylation of Bad. Importantly, BFT treatment induced 2 fold increase in proliferation of CPCs that was markedly attenuated by DM (P<0.01). In addition BFT also increased the differentiating ability of CPCs into cardiomyocytes, as evidenced by measuring the percent of cells positive for α-sarcomeric actin and connexin-43. Finally, CD45$^-$ CD90$^+$CD105$^+$ CPCs isolated from atrial appendages of patients undergoing on-pump by pass cardiac surgery were exposed to high glucose (30μM) with or without BFT (150μM). Importantly, high glucose induced apoptosis (55±5% vs 12±4%, P<0.001) and reduced the proliferation (45±8% vs 80±4%, P<0.001) and differentiation potential of human CPCs, with these effects being prevented by BFT (P<0.01 for all comparisons).
Conclusion: Both DM and high glucose cause quantitative and functional deficits in CPCs. BFT contrasted these detrimental effects in a murine diabetic model as well as in *in vitro* assays using human CPCs exposed to high glucose. These data highlight the direct protective action of BFT on CPCs and potential underpinning mechanisms centred on survival Pim-1/Bcl-2 signalling pathway. Thus, BFT merit attention as a global therapeutic target to combat DM-associated cardiac damage.
Supported by: UK Diabetes

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Gene silencing of Toll-like receptor attenuates myocardiac apoptosis in diabetic mice

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Background and aims: Myocardiac apoptosis is an early event involved in the process of cardiomyopathy in diabetes mellitus. Toll-like receptors (TLR) signaling triggers cell apoptosis through multiple mechanisms. Up-regulation of TLR4 expression has been shown in diabetic hearts. This study aims to delineate the role of TLR4 in cardiac apoptosis, and to block this process through gene silencing of TLR4 in diabetic hearts.
Materials and methods: The C57/BL6 mice were made diabetic by ip injection of streptozotocin (STZ, 150mg/kg body weight). Three days after STZ treatment, the diabetes was confirmed by blood glucose level. Diabetic mice were treated with 50 μg of TLR4 shRNA (treatment group) or scrambled shRNA (control group). Other control groups include non-treated diabetic mice (diabetes group) and naïve mice (normal group).
Results: After 7 days of hyperglycemia, the level of TLR4 in the heart tissue was significantly elevated in the diabetic mice, as comparing normal mice. Treatment of TLR4 shRNA knocked down the gene expression as comparing control mice, as well as diminished its elevation in diabetic mice. Pathologically, apoptosis was evident in the cardiac tissues of diabetic mice, as detected by TUNEL assay. In contrast, treatment with TLR4 shRNA minimized apoptosis in heart tissues. Mechanistically, caspase 3 activation was remarkably inhibited in the mice that were treated with TLR4 shRNA, but not in the mice treated with control shRNA or non-treated mice. Furthermore, gene silencing of TLR4 resulted in suppression of apoptosis cascades, such as caspase 3, caspase 8 and Fas gene expression.
Conclusion: In summary, we demonstrated here a piece of novel evidence that TLR4 plays a critical role in cardiac apoptosis. This is the first demonstration of preventing in cardiac apoptosis in diabetic mice through gene silencing of TLR4 gene.
Supported by: LHRI

PS 134 Liver, lungs and bone

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Prevalence of glucose intolerance in patients with chronic hepatitis C

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Background and aims: The association of glucose intolerance with chronic hepatitis C (CHC) virus infection remains controversial. The aim of this study was to evaluate the prevalence of glucose intolerance by oral glucose tolerance test (OGTT) in patients with CHC in comparison with matched controls and to analyze if adipocytokines levels correlate with insulin secretion (IS) and insulin resistance (IR).
Materials and methods: 176 consecutive outpatients with CHC (group A) and 123 subjects individually (group B) matched for age, sex and body mass index (BMI) were included. OGTT was performed in all cases with HbA1c over 5.5%. Glucose intolerance was defined as IFG (impaired fasting glucose), IGT (impaired glucose tolerance) or diabetes. IR was determined using Homeostasis model assessment (HOMA-IR). The liver fibrosis was non-invasively assessed using the Forns index; a value < 4.2 excludes liver fibrosis and a value > 6.9 is a predictor for significant fibrosis.
Results: The average age was 56.21±10.28 in group A and 54.27±10.22 years in group B. After age and BMI adjustment, patients with CHC had significantly higher prevalence of glucose intolerance (23.86% vs 9.75%, P = 0.037). Median HOMA-IR (3.75 versus 1.23), adiponectin (6.72 versus 2.98 μg/ml), TNF alpha (2.79 versus 0.61 pg/ml), IL-6 (4.78 versus 1.81 pg/ml) were significantly higher in CHC patients (all p < 0.05). In patients with CHC, by multiple linear regression, independent predictors of HOMA-IR included the body mass index, apparent liver disease duration, and the serum levels of leptin, TNF alpha, IL-6 (positive correlation) and adiponectin (negative correlation). In patients with HCH infection, plasma insulin and C-peptide levels were lower (al p <0.05).
Conclusion: In hepatitis C patients**,** higher prevalence of glucose intolerance are present. Adipocytokines and inflammatory cytokines play an important part in this relationship. Screening for diabetes (HbA1c or OGTT) is necessary in patients with hepatitis C.
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Prevalence and determinants of diabetes mellitus in Dutch patients with liver cirrhosis

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Background and aims: The reported prevalence of type 2 Diabetes mellitus (DM2) in patients with liver cirrhosis is 20-40%, about five times higher than in the general population. However, these data were never adjusted for classical risk factors for DM2, like age, body mass index (BMI) or family history of DM2 (first or second degree relatives). We therefore investigated the association between cirrhosis and DM2 controlling for known risk factors for DM2.
Materials and methods: We reviewed medical files for presence of DM2 and potential confounders in 94 patients with cirrhosis visiting our hospital between 2001 and 2009 (cases) and compared these to a control group of 107 patients with non-ulcer dyspepsia (NUD). Multiple logistic regression analysis was used to adjust for potential confounders.
Results: The etiology of our cirrhosis population was alcohol (59%), viral hepatitis (10%), biliary cirrhosis (3%) or cryptogenic (28%). Prevalence of DM2 was significantly higher in patients with cirrhosis than in controls: 35/94 (37%) vs. 7/107 (7%) (OR 8.5, 95%CI 3.5 - 20.2, p < 0.001). After adjustment for age, sex, family history of DM2, alcohol use and BMI, cirrhosis remained significantly associated with DM2 (OR 13.6, 95% CI 4.3 - 42.9, p < 0.001). Most DM2 was already diagnosed before diagnosis of cirrhosis (21/35, 60%) or was incidentally found together with cirrhosis (5/35, 14%). In multivariate analysis in patients with cirrhosis, male sex (OR 4.6, 95% CI 1.3 - 15.9, p = 0.016), positive family history of DM2 (3.7, 95% CI 1.0 - 12.8, p = 0.042) and BMI (OR 1.2 per kg/m^2, 95% CI 1.0 - 1.3, p = 0.015) were positively associated with DM2, whereas alcohol consumption protected (moderate vs. no alcohol consumption (OR 0.11, 95% CI 0.02 - 0.57, p = 0.009), excessive vs. no alcohol consumption (OR 0.14, 95% CI 0.03 - 0.63, p = 0.010)). Etiology and severity of the cirrhosis, expressed in Child-Pugh score, were not associated. The final model explained about 30% of diabetes prevalence in cirrhosis.
Conclusion: Liver cirrhosis was strongly associated with DM2, even after adjustment for age, BMI and family history of DM2. Although sex, family history, BMI and alcohol consumption were significantly associated with the presence of DM2, this only explained part of the high DM2 prevalence in cirrhosis. Therefore a liver-specific factor, such as low-grade inflammation, might cause DM2 in cirrhosis.

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Chromatin structure and Sirt1 and PGC-1alpha expressions in liver cells of NOD mice

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Background and aims: Evidences point out that hyperglycemia caused by diabetes mellitus is the main factor that contributes to early-ageing in diseased patients. This metabolic alteration could lead to changes in chromatin structure and dynamics, as those previously observed in hepatocytes of a group of NOD mice presenting glycemia higher than 600 mg/dl. Alterations in chromatin structure caused by epigenetic factors may change the expression profiles of genes involved in cell ageing. Sirt1 is a NAD^+ dependent deacetylase recently related to cellular metabolic changes and ageing. Together with the transcriptor factor PGC-1α, Sirt1 could act as a nutritional sensor. The aim of this study was to compare chromatin accessibility to micrococcal nuclease (MNase) and expressed Sirt1 and PGC-1α protein in liver cells of moderate and severe diabetic adult and non-diabetic mice.
Materials and methods: Young adults (8 weeks old) with moderate diabetes (glycemia: 200-400 mg/dl), severe (glycemia > 500 mg/dl) diabetic adults and old non-diabetic (56 weeks old) mouse groups were used (n = 5). Normoglycemic Balb/c and NOD mice with the same ages as the diabetic animals were used as controls. Chromatin accessibility was evaluated by Mnase assay, followed by DNA extraction and agarose gel electrophoresis. PGC-1α and Sirt1 were determined by Western Blot for the same groups.
Results: The chromatin in mice with severe diabetes presented the highest unraveling degree (2.62 ± 0.44, $P_{<0.05}$) in comparison with that of the control with the same age (Balb 0.66 ± 0.22; NOD 0.46 ± 0.11). Although chromatin accessibility index of the older mice was higher in comparison with that of young mice (1.41 ± 0.2 vs 0.67 ± 0.18, $P_{<0.05}$), it was still lower than that in mice with severe diabetes (2.62 ± 0.44, $P_{<0.05}$). The Sirt1 protein was abundant in mice with severe diabetes compared to their respective controls (moderate diabetic mice 1.62 ± 0.17 vs control Balb/c 0.92 ± 0.07, $P_{<0.05}$; severe diabetic mice 1.60 ± 0.18 vs control Balb/c 0.94 ± 0.09 and normoglycemic NOD 0.75 ± 0,09 $P_{<0.05}$) while old mice presented the lowest values (0.30 ± 0.03) in comparison to young mice (1.00) ($P_{<0.05}$). The PGC-1α abundance was higher than that of controls only when considering mice with severe diabetes (2.15 ± 0.4) vs Balb/c mice (0.66 ± 0.06) and normoglycemic NOD (0.88 ± 0.42). Similar to the Sirt1 expression, PGC-1α abundance was lower (0.51 ± 0.15 fold) in older mice in comparison with young ones.
Conclusion: Severe hyperglycemia induces enhancement of an open form of chromatin in hepatocytes, surpassing the phenotype exhibited in old specimens. Sirt1, together with PGC-1α, could act as nutritional sensor, because it is highly expressed in severe diabetic mice but attains normal levels in normoglycemic mice of same age. Therefore, the decondensed chromatin phenotype observed in hepatocytes of mice with severe diabetes is not assumed to be affected by Sirt1, as this protein, when acting as histone deacetilase, would lead to a more compact state of the chromatin structure. Considering that the expressions of Sirt1 and PGC-1α in old mice differed from diabetic adult mice, we suggest that the early ageing observed in diabetic individuals does not follow the same metabolic pathway as natural ageing.
Supported by: FAPESP and CNPq

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Effects of early undernutrition on the insulin sensitivity and on both glucose and ketone body transporters in the liver from suckling rats

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Background and aims: The suckling imposes biochemical adaptations to nutrition, since milk is a high-fat low-carbohydrate diet. As well, this period is a critical window of growth for the brain, which accomplishes part of its development before weaning. Immature brain uses large amounts of glucose and ketone bodies to obtain energy and for biosynthesis, thus increased rates of hepatic gluconeogenesis and ketogenesis are compulsory. Consistently, plasma insulin and glucagon remain in low and high concentration respectively, throughout the suckling, adapting liver metabolism to diet. What is more, hepatic low insulin responsiveness is the most suitable condition during this period. The shortage of nutrients during development can have grave consequences later in life. Impaired glucose tolerance, diabetes and cognitive defects can arise from early undernutrition. As previously shown, insulin responses are enhanced in heart, skeletal muscle and adipose tissue of restricted rats, but the effects on liver sensitivity has not been explored yet. Changes in hepatic insulin sensitivity might alter the rate of the indicated metabolic pathways, modifying the production of endogenous substrates. Our aim is to search whether maternal undernutrition influences the insulin responses in the liver of suckling pups.
Materials and methods: Wistar rats were submitted to a 60% restriction of a commercial diet from 14th day of pregnancy and during lactation. 10-day old rats from restricted dams and their controls were studied. Rats were decapitated. Blood was got from the neck. Liver was rapidly frozen. Plasma insulin and glucagon were determined by RIA. Glycogen was quantified with amyloglucosidase and ketone bodies spectrophotometrically. Liver gluconeogenesis was evaluated by a glycerol test. Components of insulin signalling were analyzed by Western. IRS-1/2 associated PI3-kinase was assayed with [^{32}P]ATP.
Results: Glycaemia was depressed in the restricted rats. Blood and liver ketone bodies remained above control values. Undernutrition decreased plasma insulin and glucagon. Liver glycogen was enhanced. Glycerol gluconeogenesis did not change, nor liver expression of insulin receptor, but IRS-1/2 increased with undernutrition. PI3-kinase was more activated by insulin in restricted rats, as was Akt and GSK3 phosphorylation.
Conclusion: Early undernutrition increases the risk for defects in glucose homeostasis and for neurological damages. Herein we show that undernourished rats are hypoglycaemic, due to the liver insulin hypersensitivity (as in other tissues) together with a low plasma glucagon. These changes mean a dramatic modification of the most convenient hepatic metabolic setting during suckling. The high ketogenic ability in restricted animals is rather surprising, considering the enhanced insulin responsiveness. The improved plasma levels of ketone body probably constitutes an adaptation to nutritional restriction. It might prevent or minimize the impact on immature organs, as the brain. However, glucose is required for normal brain functioning under most conditions, including perinatal time. So imbalance between plasma glucose and ketone body concentrations might have deleterious consequences for the developing brain.
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The morphometric characteristics of pulmonary tissue and arteries in type 2 diabetic patients

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Background and aims: Diabetes mellitus is associated with the damage of different organs. However, the changes of the lungs in patients with diabetes are not fully characterized. Therefore, the aim of our study was to investigate the morphometric characteristics of an alveolar tissue and pulmonary arteries in type 2 diabetic patients.
Materials and methods: We studied the materials obtained from the autopsy of 25 deceased subjects with type 2 diabetes mellitus (mean age - 58,3±1,3 years) and 11 deceased persons without diabetes (mean age - 50,2±1,6 years) as the control group. The causes of death in those subjects were not related to pulmonary disease. The tissue samples were sectioned and fixed in 10% neutral formol by standard histological methods and embedded in paraffin wax. The tissue slides were stained with hematoxylin-eosin. We analyzed the morphometric characteristics of pulmonary tissues and arteries using the light-optical microscope (Olympus, Japan, BX - 41).
Results: We found the trend toward an increase of the lumen of alveoli in those with diabetes compared to controls - 137,2±10,8 μm vs. 101,2±12,6 μm, respectively, 0,05<p<0,1 (data are presented as mean±SEM). These changes were accompanied by the significant decrease of the external diameter (100,8±1,6 μm vs. 172,3±27,4 μm, (p<0,001) and internal diameter (76,03±5,5 μm vs. 111,3±18,6 μm, (p<0,001) of pulmonary arteries in diabetic subjects compared to controls. Moreover, we found the significant decrease of the thickness of the arteries wall in those with diabetes (12,3±0,53 μm vs. 35,6±4,9 μm, (p<0,001), the vascular index was 14,3±0,42 vs. 22,6±0,97, (p<0,001), in those with and without diabetes, respectively.
Conclusion: We may conclude that the revealed morphometric changes of alveolar tissue and arteries in type 2 diabetic patients may underlie the higher susceptibility to pulmonary diseases in these patients.
Supported by: OAP

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Serum levels of bone turnover markers in type 2 diabetes and their relationship with bone mineral density

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Background and aims: Several studies indicate that hyperglycemia induces a low turnover state with osteoblast dysfunction. Studies of bone resorption in diabetes are limited, and the results are conflicting. Our aim was to analyse serum levels of bone turnover markers (BTM), PTH-i and 25 OH vitamin D in patients with type 2 diabetes mellitus (T2DM), and the relationship with bone mineral density (BMD). We also compared BTM between T2DM and controls.
Materials and methods: Case-Control study including 133 subjects, 78 patients with T2DM and 55 healthy controls. Lumbar spine and femoral BMD were measured by dual X-Ray absorptiometry (Hologic QDR 4500). We measured: bone alkaline phosphatase (b-ALP) by an ELISA (OCTEIATM IDS Ltd Boldon UK), osteocalcin (OC) by radioimmunoassay (DiaSorin, Stillwater, Minnesota USA; TRAP (Bone TRAP ® Assay. IDS Ltd); CTX by ELISA (Elecsys ß CrossLaps,Roche Diagnostics SL, Barcelona, Spain); PTH-i (Intact PTH, Roche Diagnostics SL); 25 OH vitamin D (25-Hydroxyvitamin D 125I RIA DiaSorin).
Results: Mean age was 56,7± 6,8 yr (57.8± 6.4 and 55,1± 7,1 in T2DM and control group respectively; p=0.024). Among the T2DM patients (n=78), 47.2% were females (n=35) and 52.8% males (n=43). In the control group 56.5% were females (n=30) and 43.5% males (n=25). Serum levels of bone resorption markers were lower in T2DM compared with controls (TRAP: T2DM 1.39±0.99 UI/l vs controls 1.85±0.81 UI/l, p<0.05; CTX: T2DM 0.20±0.12 ng/ml vs controls 0.33±0.15 ng/ml, p<0.05). There were no differences in bone formation markers (b-ALP: T2DM 14.83±6.5 ug/L vs controls 12.96±6.73 ug/L, p 0.11; OC: T2DM 1.48±1.25 ng/ml vs controls 1.45±1.2 ng/ml, p 0.91). PTH-i serum levels were lower in T2DM (PTH-i: T2DM 38.35±18.20 pg/ml vs controls 50.22±18.99 pg/ml, p<0.05). T2DM have lower levels of 25 OH vitamin D with respect to controls, although differences were not significant (T2DM 17.81±11.14 ng/ml vs controls 21.30±11.05 ng/ml, p 0.07). In T2DM there was a negative correlation between CTX levels and BMD at different sites (LS BMD -0.460, P<0.001; TF BMD -0.530, p<0.001).
Conclusion: T2DM patients have lower levels of bone resorption markers and PTH-i compared with controls. CTX serum levels were negatively correlated with BMD at different sites.

PS 135 Steatohepatitis

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Predictors of impaired glucose regulation in patients with non-alcoholic fatty liver disease

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Background and aims: Many patients with non-alcoholic fatty liver disease (NAFLD) have impaired glucose tolerance or type 2 diabetes mellitus (DM). We sought to identify characteristics of NAFLD patients associated with hyperglycemia.
Materials and methods: We prospectively studied a cohort of NAFLD patients. They underwent a 2-hour oral glucose tolerance test (using 75 g). Serum glucose and insulin were measured at 30 min intervals. Common biochemical laboratory tests were also done.
Results: We included 152 patients with NAFLD; 74 (48.7%) had hyperglycemia (45 had impaired fasting glucose or impaired glucose tolerance, and 29 had type 2 DM). Patients with hyperglycemia had higher body mass index (BMI; 30.5±4.5 vs. 28.5±4.8 kg/m2, p=0.01), lower high-density lipoprotein cholesterol (HDL-C; 46.5±13.6 vs. 53.7±18.8 mg/dL, p=0.02), lower serum albumin (4.1±0.5 vs. 4.4±0.4 g/dL, p<0.01) and were older (53.0±10.7 vs. 47.3±11.4 years, p<0.01), in comparison with patients with normoglycemia. In multivariate analysis including the above 4 variables as covariates, age (odds ratio [OR]: 1.08, 95% confidence interval [CI]: 1.03-1.13), BMI (OR: 1.12, 95% CI: 1.01-1.25), and HDL-C (OR: 0.95, 95% CI: 0.92-0.98) proved independent predictors of hyperglycemia in NAFLD patients. Additionally, 30-min insulin after the oral glucose challenge was lower in patients with hyperglycemia (74.2±49.7 vs. 94.5±53.9 μIU/ml, p= 0.02), while 90-min insulin (170.1±84.6 vs. 122.9±97.7 μIU/ml, p=0.01) and 120-min insulin (164.0±101.2 vs. 85.3±61.9 μIU/ml, p<0.01) were higher and insulin at 60-min did not differ between patients with or without hyperglycemia.
Conclusion: NAFLD patients with higher BMI, lower HDL-C, or older age were more likely to have impaired glucose metabolism. We suggest that oral glucose tolerance test should be considered for patients with non-alcoholic fatty liver disease, particularly in those with one or more with the above mentioned predictor characteristics of hyperglycemia, to readily diagnose and treat disorders of glucose metabolism.

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Non-alcoholic fatty liver disease in patients with type 2 diabetes mellitus

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Background and aim: Non-alcoholic fatty liver disease (NAFLD) is a growing medical problem worldwide. It has gained considerable attention for its appealing relations with insulin resistance and the slow-evolving course towards the end-stage liver disease. We conducted a prospective study of incidence and risk factors of NAFLD in patients with type 2 diabetes mellitus (DM).
Methods: 127 consecutive patients with type 2 DM [60 (47.2%) males and 67 (52.8%) females, mean age 59.3±9.3 years old] hospitalized in University Service of Endocrinology in Tirana, Albania during 2009 and fulfilling diagnostic criteria adopted by WHO 1999 were included in the study. The diagnosis of NAFLD was based on 1) sonographic findings, 2) ethanol intake equal or lower than 20 g/day and 3) exclusion of all other liver diseases. The clinical and laboratory parameters, such as age, sex, mean duration of diabetes, body mass index (BMI), glycosylated hemoglobin (HbA1c), transaminases levels, serum cholesterol and triglyceride levels were analyzed as predictors of sonographic findings by using multiple logistic regression model.
Results: 74% (N=94) of the patients had NAFLD [39/94 (41.5%) mild, 45/94 (47.9%) moderate, 10/94 (10.6%) severe]. The value of BMI, WHR (waist-hip ratio), ALT (alanine aminotransferase), serum cholesterol and triglyceride in type 2 DM patients with NAFLD were significantly higher than in those without NAFLD [26.5 vs. 33.9 kg/m^2, 0.91 vs. 1.12, 26.8 vs. 40.19 (UI/L), 193.9 vs.230.1 (mg/dl) and 141.7 vs. 227.9 (mg/dl), respectively, P<0.001). All patients (N=13; 14%) with ALT greater than normal had NAFLD. In multivariable-adjusted models significant predictors were age (OR=1.04, 95% CI=0.03-0.85), BMI (OR=1.53, 95% CI=0.10-0.32) and HbA1c (OR=1.1, 95% CI=0.11-0.23).
Conclusion: In Albania, the incidence of NAFLD seems to be higher in patients with type 2 diabetes mellitus. Older age, obesity and HbA1c were the independent risk factors of NAFLD in diabetic patients.

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High prevalence of advanced NAFLD in type 2 diabetic patients with unremarkable liver enzymes and effect of liraglutide on NAFLD: meta-analysis of the LEAD Program

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of liver disease in type 2 diabetes. Predicting patients at risk of progressing to the advanced stages of NAFLD (liver inflammation and fibrosis) is a clinical challenge as the majority of patients are asymptomatic and have normal liver enzyme levels. The NAFLD Fibrosis Score (NFS) is a well-validated non-invasive scoring system (comprising age, BMI, AST:ALT ratio, platelets and albumin) used to predict the severity of advanced liver fibrosis. The aims of this study are: (i) to estimate the severity of NAFLD using the NFS and (ii) to evaluate the effect of 1.8mg liraglutide, a once-daily human GLP-1 analogue, on NAFLD in a cohort of poorly controlled diabetic subjects.
Materials and methods: Meta-analysis was performed on individual data from patients enrolled in the Liraglutide Effect and Action in Diabetes (LEAD) program. ANCOVA analysis was performed on the intent-to-treat population to estimate change from baseline after 26 weeks.
Results: Data from 3967 adults at baseline: 53% male; 79% Caucasian; Age 56 years [10]; HbA1c 8.3% [1.0]; duration of diabetes 7.8 years [5.8]; systolic BP 131 [15.3]; BMI 31.6 kg/m2 [5.40]; males ALT 32 and females ALT 24 IU/L (*values expressed as mean [SD]*). 70% of subjects had metabolic syndrome based on ATP III classification. 54% of subjects had abnormal ALT (upper limit of normal range 30 IU/L for males and 19 IU/L for females), with mean ALT 39 IU/L. The NFS predicted that 6.4% had advanced liver fibrosis (score > +0.676) and 61.0% had an indeterminate score (-1.455 to 0.676) requiring further liver specialist review. Duration of diabetes significantly correlated with ALT (r_s = 0.17, p<0.0001) and severity of NFS (r_s = 0.14, p<0.0001). Liraglutide significantly reduced ALT versus placebo (LS mean 3.48 vs 1.36; p<0.001), although the proportion of patients normalizing their ALT was similar (25.2% vs 22.7%). Liraglutide significantly improved NFS in comparison to placebo (LS mean -0.40 vs -0.13; p<0.0001), with significant changes in BMI (LS mean -0.66; p<0.0001) and platelet count (LS mean +15.90; p<0.0001). There was no relationship between changes in NFS and HbA1c after 26 weeks liraglutide treatment (r_s = 0.00, p 0.96).
Conclusion: Advanced NAFLD fibrosis is present in a significant proportion of diabetic patients in the LEAD program, despite the absence of clinically significant serum transaminases. Duration of diabetes, but not level of glycaemic control was correlated with elevation of ALT and worsening severity of NFS. 26 weeks treatment with Liraglutide reduces ALT and NFS. The increase in platelets after Liraglutide treatment may indicate an effect independent of weight loss in NAFLD.
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Insulinaemic status and nonalcoholic fatty liver disease in Bangladeshi type 2 diabetic subjects

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Background and aims: Nonalcoholic fatty liver disease (NAFLD) is becoming a public health problem with increasing incidence and it has been shown to be associated with T2DM and other disorders of metabolic syndrome

(MS)). Insulin resistance (IR) is a central feature of both T2DM and MS, but the association of IR with NAFLD has yet not been fully clarified taking into account the racial heterogenecity and confounders like BMI. Moreover, the pathogenesis of T2DM Bangladeshi subjects has pancreatic β cell dysfunction as a predominant defect and its association with NAFLD needs to be investigated. The issue was addressed in this study through exploring the association of NAFLD with insulin secretion and sensitivity as measured by homeostasis model assessment.
Materials and methods: Under a Case-control design this analytic observational study was conducted on 127 T2DM with NAFLD and 129 T2DM without NAFLD subjects. T2DM was diagnosed by WHO Study Group Criteria and NAFLD was diagnosed by 4D ultrasound. Insulin was assayed by an ELISA technique, and insulin secretory capacity (HOMA% B) and insulin sensitivity (HOMA% S) were estimated by homeostasis model assessment using a HOMA-SIGMA software. Data were analyzed by appropriate univariate as well as multivariate tests including logistic regression analysis with NAFLD as dependent variable and others as independent/confounding variables as appropriate.
Results: The NAFLD group was found to have higher BMI as compared to the Control (BMI, M± SD, 26.19± 3.69 vs 25.32± 3.47, p= 0.053); however, no significant difference was observed in case of WHR. The fasting blood glucose and lipid levels did not differ between the two groups. Serum fasting insulin was significantly higher in case of NAFLD group [fasting serum insulin in IU/L, of NAFLDr vs Non-NAFLD, Median (Range), 19.8(3.0-81.6) vs 16.67(3-100.6), p=0.005]. HOMA%B was significantly higher in NAFLD group compared to T2DM subjects [HOMA%B, NAFLD vs Non-NAFLD, 108.9 (6.30-462.70) vs 90.8 (11.1-451.3), p=0.014]. In case of insulin sensitivity a significant difference was present between NAFLD and Non- NAFLD groups [HOMA%S, NAFLD vs Non-NAFLD, 31.9(5.8-212.10) vs 34 (6.8-190.8), p=0.005]. In logistic regression analysis, taking fatty liver as the dependent variable, and WHR, BMI, FBG, HOMA%S and HOMA%B as independent variables a significant negative association of fatty liver was found with HOMA% S (p= 0.033).
Conclusion: The data suggest that NAFLD is associated with insulin resistance in Bangladeshi T2DM subjects, and the association of IR and NAFLD is independent to obesity and glycemic- insulinemic status.
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Alpha-lipoic acid attenuates steatohepatitis through inhibiting CYP2E1 and endoplasmic reticulum stress

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Background and aims: Nonalcoholic fatty liver disease (NAFLD) is closely associated with obesity, and type 2 diabetes. In the insulin-resistant state, increased hepatic fat accumulation and reactive oxygen species (ROS) stimulates the activity of CYP2E1 and endoplasmic reticulum (ER) stress. Here, we examined whether alpha-lipoic acid (ALA) prevents steatohepatitis through the inhibition of molecular mediators involved in hepatic injury including CYP2E1 and ER stress.
Materials and methods: C57BL/6 mice were fed a methionine choline deficient (MCD) diet with or without ALA for 4 weeks. The plasma levels of ALT and AST were measured to check hepatic inflammation. For histological analysis, we conducted hematoxylin and eosin staining and Oil red O stating. We investigated the effect of ALA on CYP2E1 and ER stress in the liver of MCD-diet mice using Northern blot analysis and Western blot analysis.
Results: Dietary supplementation with ALA reduced MCD diet-induced hepatic lipid accumulation, hepatic inflammation and plasma ALT and AST levels. Upon histological examination, the livers of MCD mice exhibited steatohepatitis, including fat accumulation and infiltration by inflammatory cells. Mice fed an MCD diet supplemented with ALA exhibited significantly attenuated hepatic fat accumulation and inflammation. CYP2E1 expression in the liver of MCD mice was significantly higher than that of control mice, and ALA inhibited the MCD diet-induced expression of CYP2E1 at the mRNA and protein levels. Moreover, ALA inhibited MCD diet-induced expression of the nuclear factor-E2-related factor 2 (Nrf2) and Nrf2 target gene NAD(P)H quinone oxidoreductase 1. MCD diet increased the activation of ER stress markers, such as phosphorylation of eIF2α and expression of GRP78, ATF6, and CHOP, and also induced an increase in levels of MAP kinase activity, including JNK and ERK phosphorylation. MCD diet fed mice with ALA attenuated these expressions.
Conclusion: Taken together, the results of the present study indicate that ALA attenuates steatohepatitis through inhibition of CYP2E1 and ER stress. It provides the possibility that ALA can be used to prevent the development and progression of NAFLD in patients who have strong risk factors for NASH.

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Specifically-PNPLA3-mediated accumulation of liver fat in type 2 diabetic patient

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is commonly associated with obesity, metabolic syndrome and type 2 diabetes. Recently, it has been shown in the general population that an allele in the adiponutrin (PNPLA3) gene (rs738409[G]) was strongly associated with increased hepatic fat levels, independently of visceral adiposity and insulin resistance. In this study, we set out to determine whether liver fat content (LFC), evaluated using ^{1}H-MR Spectroscopy, was associated with PNPLA3 rs738409 polymorphism in people with type 2 diabetes. We also evaluated the influence of this polymorphism on the relationship between LFC and either visceral adiposity or intima media thickness (IMT).
Materials and methods: 218 type 2 diabetic patients were included in this study. LFC (1H-magnetic resonance spectroscopy), area of visceral fat (RMN), and IMT were measured.
Results: 139 (63.7 %) patients had steatosis. Patients with steatosis had a higher BMI (34.9 +/- 6.3 vs 32.9 +/- 6.6; p= 0.01), higher visceral fat area (284 +/- 94 vs 246 +/- 100 cm2, p= 0.005), higher plasma ALAT levels (42.8 +/- 22.5 vs 31.6 +/- 36.7 UI/l, p< 0.001), and higher plasma triglyceride levels (2.42 +/- 1.77 vs 1.78 +/- 1.05 mmol/l, p= 0.004) than did patients without steatosis. The rs738409 minor G allele was associated with LFC. The number of patients with steatosis is significantly higher among minor G allele carriers in comparison to C allele homozygote carriers (70.3% vs 57.2%; p=0.04) In the sub group of C allele homozygote carriers, LFC correlated with BMI (r=0.27, p=0.003), and visceral fat area (r=0.30, p=0.002) but not with IMT. In the sub group of minor G allele carriers, LFC correlated negatively with IMT (r=-0.23, p=0.03), but not with BMI, or with visceral fat area. Circulating triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, AST and ALAT levels were similar for minor G and C allele homozygote carriers.
Conclusion: This study suggests that in people with type 2 diabetes, LFC is related to rs738409 polymorphism. Moreover, an interesting finding of this study was that the relationship between metabolic factors and LFC were very different in each subgroup of the rs738409 polymorphism. The lack of a relationship with visceral obesity and the negative correlation with IMT suggest that fatty liver associated with the minor G allele of the PNPLA3 rs738409 polymorphism may not be linked to metabolic disorders. The reason why liver fat content is negatively associated with carotid atherosclerosis among PNPLA3-minor G allele carriers is not yet known. The possibility that in such patient, hepatic triglyceride synthesis could protect other tissues from the potential lipotoxicity of free fatty acids needs to be evaluated.

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